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Mayke B. Hesselink · Chris G. Parsons
Christina Wollenburg · Wojciech Danysz

Brain distribution of an uncompetitive NMDA receptor antagonist; comparison to its in vitro potency in electrophysiological studies

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Abstract Although the concentration of drugs in brain homogenates is relatively easy to determine, such data are sometimes misleading due to accumulation in intracellular compartments. This is apparent for uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists where concentrations assessed in this manner are much higher than those sufficient to block the NMDA channel from the extracellular space. The aim of the present study was to determine whether free brain concentrations (extracellular fluid – ECF) of a new uncompetitive NMDA receptor antagonist MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride) following administration of doses effective in animal models are sufficient to block NMDA receptors based on its potency in vitro. This issue was addressed using brain microdialysis corrected for in vivo recovery and patch clamp experiments. MRZ 2/579 blocked steady-state inward current responses of cultured hippocampal neurones to NMDA with an IC_{50} of 1.11 μ M at -70 mV. Much higher concentrations of MRZ 2/579 blocked voltage-activated Ca^{2+} channels with an IC_{50} of 340 μ M. MRZ 2/579 (10 μ M) reduced peak inward current responses of neuronal nicotinic receptors only to 72.3% of control. MRZ 2/579 (10–100 μ M) had little or no effect at AMPA and $GABA_A$ receptors. Following chronic s.c. infusion of MRZ 2/579 (40 mg/kg day for 7 days) brain ECF (2.15 μ M) and cerebro-spinal fluid (CSF) levels (2.16 μ M) were twofold lower than free plasma levels (4.3 μ M). MRZ 2/579 showed pronounced accumulation in brain tissue compared to free plasma (28-fold) and ECF (58-fold). After acute i.p. administration (5, 10 and 20 mg/kg) peak concentrations in ECF were 0.70, 0.96 and 2.53 μ M, respectively. In conclusion, MRZ

2/579 is indeed strongly accumulated in brain tissue compared to brain ECF, CSF and plasma. However, the brain ECF levels attained following administration of behaviourally effective doses are sufficient for selective NMDA receptor blockade.

Key words MRZ 2/579 · In vivo recovery · Microdialysis · Patch clamp · Uncompetitive NMDA receptor antagonist · Zero-net flux

Introduction

Antagonists of the NMDA receptor may potentially be used in a wide variety of therapeutic applications (for reviews see Danysz et al. 1995; Small and Buchan 1997). Uncompetitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptor bind to the channel site in a 'use-dependent' manner. This means that they usually only block the channel when it is in an activated and therefore open state. Moderate-affinity uncompetitive NMDA receptor antagonists with fast blocking kinetics and strong voltage-dependency show a more favourable side-effect profile than high-affinity agents such as (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate [(+)-MK-801; Chen et al. 1992; Parsons et al. 1993; Rogawski 1993; Danysz et al. 1995; Parsons et al. 1999]. An explanation for this difference could lie in the fact that the moderate-affinity NMDA receptor channel blockers still allow a certain level of physiological receptor activation during transient synaptic activity, while sustained receptor activation at pathophysiological concentrations of glutamate is blocked (Parsons et al. 1995). In fact, the aminoadamantanes memantine and amantadine have been used clinically in the treatment of Parkinson's and Alzheimer's disease, respectively, and are well tolerated (Pantev et al. 1993; Kornhuber et al. 1994; Kornhuber and Quack 1995).

Another group of uncompetitive NMDA receptor antagonists having similar features are the recently described amino-alkyl-cyclohexanes, with MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride)

M. B. Hesselink · C. G. Parsons · C. Wollenburg · W. Danysz (✉)
Department of Pharmacological Research, Merz + Co.,
D-60318 Frankfurt/Main, Germany
e-mail: wojciech.danysz@merz.de, Fax: +49-69-5962150

M. B. Hesselink
Department of Pharmacology,
Leiden/Amsterdam Center for Drug Research,
2300 RA Leiden, The Netherlands

being the most advanced in development (Parsons et al. 1999). They inhibit MES (maximal electroshock)-induced convulsions (Parsons et al. 1999) and MRZ 2/579 shows other *in vivo* effects (Popik et al. 1998; Karcz-Kubicha et al. 1999) including neuroprotection (Wenk et al. 1998), indicating, but not proving, that it reaches the CNS in sufficient concentrations to block NMDA receptors.

However, the aminoadamantanes and other uncompetitive NMDA receptor antagonists have been shown to accumulate in brain tissue as compared to plasma/serum levels (Vezzani et al. 1989; Hartvig et al. 1995; Misztal et al. 1996; Danysz et al. 1997). The present study was designed to determine to what extent MRZ 2/579 accumulates in brain tissue and whether the free brain extracellular fluid (ECF) concentration is sufficient to selectively block NMDA receptors, as determined *in vitro* in patch clamp experiments.

Materials and methods

In vitro patch clamp experiments

Hippocampal cultures were obtained from rat embryos (E20 to E21) as described previously (Parsons et al. 1995). Patch clamp recordings were made from these neurones after 12–15 days *in vitro* with polished glass electrodes (2–3 m Ω) in the whole cell mode at room temperature (20–22 °C) with the aid of an EPC-7 amplifier (List). Test substances were applied by switching channels of a custom-made fast superfusion system with a common outflow (15–20 ms exchange times). The contents of the intracellular solution were normally as follows (mM): CsCl (120), TEACl (20), EGTA (10), MgCl₂ (1), CaCl₂ (0.2), glucose (10), ATP (2), cAMP (0.25); pH was adjusted to 7.3 with CsOH, KOH or HCl. The extracellular solutions had the following basic composition (mM): NaCl (140), KCl (3), CaCl₂ (1.5), glucose (10), HEPES (10), sucrose (4.5), tetrodotoxin (TTX; 3×10^{-4}), glycine (1×10^{-3}). NB: experiments on the potency of MRZ 2/579 as an NMDA receptor antagonist were performed with intracellular K⁺ replacing Cs⁺ (cf. Parsons et al. 1999).

Only results from stable cells were accepted for inclusion in the final analysis, i.e. showing at least 60% recovery of responses following removal of MRZ 2/579. Despite this, recovery was not always complete because of rundown in some cells – for NMDA recovery responses were $85.7 \pm 4.6\%$ of control values. This rundown was more pronounced for neuronal nicotinic receptors – recovery responses were $72.9 \pm 12\%$ of control values. Rundown was always compensated by basing the % antagonism at each concentration on both control and recovery and accounting for the almost linear time course for this rundown which was confirmed in control experiments. Concentration-dependence of antagonism was always assessed at steady-state blockade with 4–6 concentrations on at least five cells.

In vivo experiments

Animals and microdialysis procedure. Adult male Sprague Dawley rats (235–275 g; Charles River, Germany) were kept under standard laboratory conditions: 12/12-h dark/light cycle, 20 °C with free access to food and water. All experiments were carried out according to project number II17a-19c20/15-F77/48 of the ethical committee of the “Regierungspräsidium Darmstadt”, Germany. All results are expressed as means \pm SEM.

For microdialysis probe implantation the animals were anaesthetised with Hypnorm (1.0 ml/kg *i.m.*; Janssen Pharmaceutical, Beerse, Belgium), placed in a stereotaxic frame, and a microdialysis guide cannula (CMA/10; CMA/microdialysis, Sweden) was

implanted in the anterior striatum relative to bregma (AP: 1.0; L: 2.5, V: –3.0). The guide cannula was attached onto the skull using screws and dental cement (Durelon; ESPE, Germany) and a microdialysis probe (3.0 mm) was inserted into the guide immediately after surgery. The animals were allowed to recover for 22–26 h.

At the start of the experiment the inflow line was connected to a syringe pump by means of a dual channel swivel (CMA) and the probe was perfused with artificial cerebro-spinal fluid (aCSF) with a flow rate of 3 μ l/min. The outlet tubing was connected to a microfraction collector and 20-min fractions were collected.

Zero-net flux method (ZNF) of determining the *in vivo* recovery. The animals used in the ZNF experiment ($n=3$) were implanted with osmotic minipumps (ALZA, Palo Alto, Calif., USA; model 2ML1) delivering ca. 40 mg/kg day MRZ 2/579 (Merz, Germany) for 7 days before implantation of the microdialysis probe to assure steady-state conditions. The microdialysis probe was continuously perfused with an aCSF solution containing different concentrations of MRZ 2/579 (0, 0.4, 2 and 4 μ M). Every inflow-concentration was perfused for 2 h before three 20-min samples were collected. By plotting the difference between C_{in} and C_{out} vs. C_{in} , the intercept with the x-axis represents the free brain ECF concentration (C_{ECF}). The *in vivo* recovery of the probe can be calculated from the slope of the linear regression line. At the end of the experiment the animals were anaesthetised. A 150- μ l CSF sample was removed from the cisterna magna (using a 27 G. needle), a blood sample (5 ml) was taken by heart puncture and the brain was removed. Parts of the plasma from the blood sample were ultrafiltered to establish to what extent the drug was bound to plasma protein (ultrafiltration tubes MW cut-off =10,000; Centrisart, Sartorius, Germany).

Acute *i.p.* administration of MRZ 2/579. In microdialysis experiments, following 2 h of perfusion of the microdialysis probe, all animals received an *i.p.* injection of MRZ 2/579 at a dose of 5 ($n=5$), 10 ($n=4$), or 20 ($n=3$) mg/kg in saline. Microdialysate samples were collected for up to 7 h. A separate experiment was performed to assess plasma and brain pharmacokinetics. MRZ 2/579 (5, 10 and 20 mg/kg) was injected *i.p.* and blood and brain samples were collected after 15, 30 and 60 min and after 2, 4, 8 and 24 h (under Nembutal anaesthesia – 1.5 ml/kg).

Analytical methods. Brain tissue was homogenized in a 4-ml vial with a disposable spatula. Then 2 ml of 2.5 M H₂SO₄ was pipetted to 0.2 g of brain sample, mixed on the Vortex and heated for 60 min at 90 °C in the heating block. Subsequently 250 μ l of homogeneous brain, 250 μ l of ISTD solution (Amantadine-HCl about 1 mg/l H₂O), 500 μ l water and 1 ml *n*-hexane were pipetted into a 4-ml vial and extracted for 30 min on the roller mixer. After centrifugation for 10 min at 4,000 rpm, the organic phase was rejected. After that, 1 ml *n*-hexane and 0.5 ml of 10 M NaOH were pipetted to the remaining solution. This mixture was extracted on the roller mixer for 30 min. Subsequently, the vial with the mixture was centrifuged for 10 min at 4,000 rpm, the organic phase was transferred into a GC-vial and 50 μ l MBTFA was added. The sample was mixed on the Vortex mixer and heated for 30 min at 70 °C in a heating block.

Microdialysate, serum or CSF samples (50–100 μ l) were added to 250 μ l of 2 N HCl, and 250 μ l internal standard (ISTD) solution (Amantadine-HCl about 1 mg/l H₂O) was pipetted into a 4-ml vial with a screw cap and heated for 30 min at 70 °C in the heating block. After cooling to room temperature, 1 ml *n*-hexane and 0.25 ml of 10 M NaOH were added and the mixture was extracted for 30 min on the roller mixer. Subsequently, the test tubes with the mixture were centrifuged for 10 min at 4,000 rpm, the organic phase was transferred into a vial and 15 μ l MBTFA were added. After that, the sample was mixed on the Vortex mixer, and the organic phase was reduced at 70 °C to 150 μ l in a heating block. After transfer of the sample to a vial insert, the measurement was carried out with the GC/MSD (Hewlett Packard HP 5890 II with MSD 5971A/5972). Measurement conditions were as follows: column: HP1 (Hewlett Packard) Cl-methylsilicone 25 m \times 0.2 mm

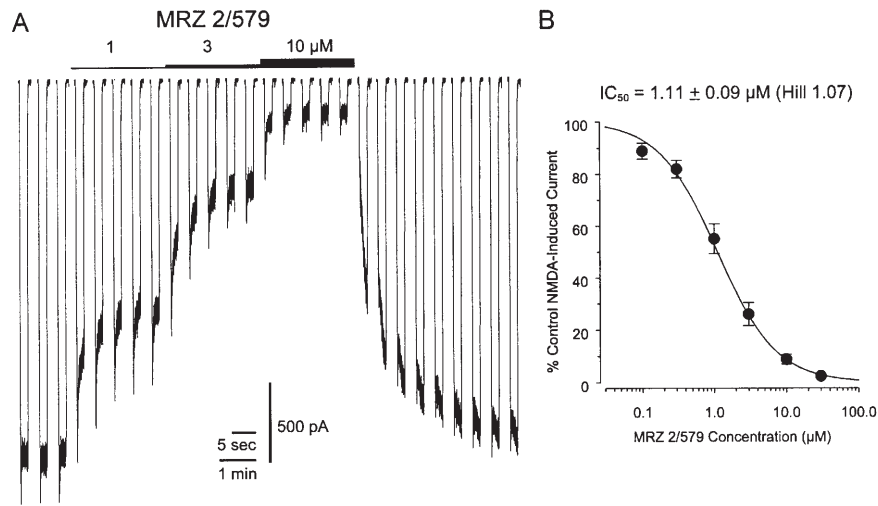


Fig. 1A Use-dependence of MRZ 2/579 on a single hippocampal neurone. NMDA (200 μM) was applied for 2.5 s every 30 s in the continuous presence of glycine (1 μM) and at a constant membrane potential of -70 mV. This inter-response interval has been omitted for clarity (*two time scales*). MRZ 2/579 (1, 3 and 10 μM) was continuously present for 2.5 min at each concentration as indicated by the graded bar. Note that the first peak response following a 15–20 s pre-incubation with each concentration of MRZ

2/579 was less affected. **B** Concentration-dependence of the blockade of NMDA receptors by MRZ 2/579. Steady-state current responses (i.e. at the end of 2.5-s NMDA applications) were normalized to control levels and plotted as means (\pm SEM) against MRZ 2/579 concentrations ($n=10$ –16 per concentration). Estimation of IC_{50} values and curve fitting were done according to the 4-parameter logistic equation (GraFit, Erithacus Software)

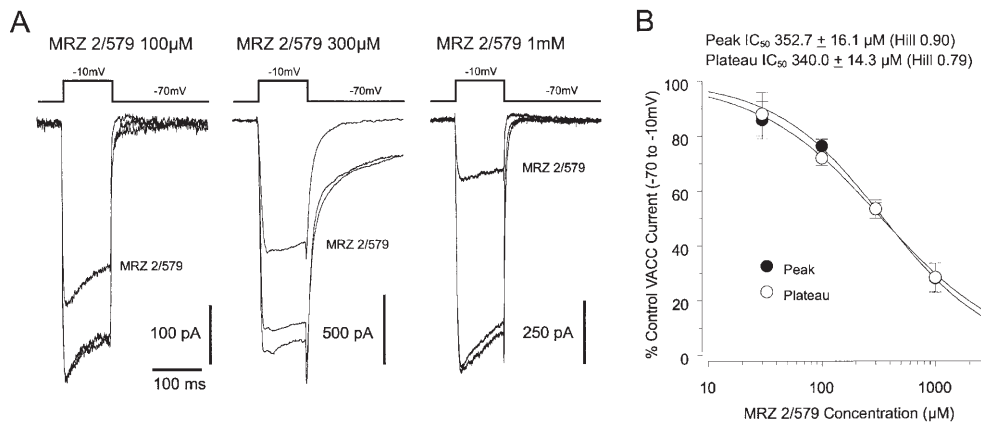


Fig. 2A, B MRZ 2/579 has weak antagonistic effects at voltage-activated Ca^{2+} channels (VACC). **A** Inward currents of three different cultured hippocampal pyramidal neurones following 100-ms voltage steps from -70 mV to -10 mV every 20 s. Each trace is the average of 3–5 consecutive responses. The largest currents are control responses and the second largest are recovery responses, i.e. there was some degree of rundown in some cells. At least 5 min were allowed for equilibrium blockade by MRZ 2/579 to develop. **B** Pooled data from cultured hippocampal neurones ($n \geq 6$ at each concentration) showing that MRZ 2/579 blocked plateau currents with an IC_{50} of 340 ± 14.3 μM (mean \pm SEM; Hill coefficient = 0.79)

ID; carrier gas: helium 12 psi; injection mode: splitless 3 μl; injection temperature: 250 °C; detector temperature: 280 °C; detected ions: TFA MRZ 2/579 137 (± 0.2 amu) and TFA Amantadine 247 (± 0.2 amu).

Results

In vitro patch clamp experiments

Potency at NMDA receptors

Steady-state inward current responses of cultured hippocampal neurones to NMDA (200 μM with glycine 1 μM at -70 mV) were antagonized by MRZ 2/579 with an IC_{50} of 1.11 ± 0.09 μM (mean \pm SEM; Fig. 1). Peak and steady-state currents were affected to a similar degree at equilibrium. Note that the first peak response following a 15–20 s pre-incubation with each concentration of MRZ 2/579 was less affected, reflecting the use-dependency of this blockade. This is also reflected in the relatively slow recovery from the effects of MRZ 2/579, taking 5–6 responses to return to control levels after removal of MRZ 2/579.

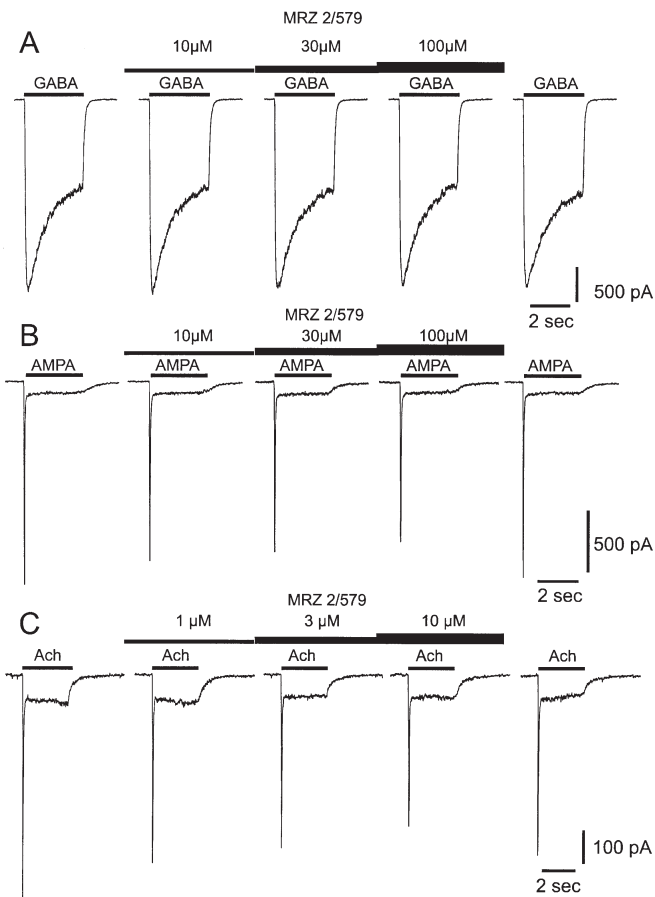


Fig. 3 A–C MRZ 2/579 has no effects at GABA_A or AMPA receptors and weak antagonistic effects at neuronal nicotinic receptors. Inward currents of three different cultured hippocampal pyramidal neurones. For each data series *left and right traces* represent control and recovery responses, respectively. The *middle three traces* represent equilibrium responses in the continuous presence of MRZ 2/579 at concentrations indicated by the *graded bar*. **A** GABA (10 μ M) was applied for 3 s every 30 s. Similar effects were seen with eight cells, and MRZ 2/579 (100 μ M) reduced plateau responses to $92.6 \pm 2.3\%$ of control. **B** AMPA (100 μ M) was applied for 3 s every 30 s. Similar effects were seen with seven cells and MRZ 2/579 (100 μ M) reduced peak responses to $90.8 \pm 2.3\%$ of control. **C** Ach (1 μ M) was applied for 2.5 s every 30 s in the continuous presence of atropine 1 μ M. NB: responses showed fast, pronounced desensitization characteristic for type 1A responses. Similar effects were seen with eight cells, and MRZ 2/579 (10 μ M) reduced peak responses to $72.3 \pm 2.9\%$ of control with no effect on plateau responses ($95.9 \pm 3.6\%$ of control). The same concentration of MRZ 2/579 (10 μ M) reduced plateau responses to NMDA to $9.0 \pm 0.9\%$ of control ($n=10$)

Selectivity of MRZ 2/579

It might be predicted that moderate-affinity uncompetitive NMDA receptor antagonists also block other Ca²⁺-permeable channels. We therefore investigated the effects of MRZ 2/579 on voltage-activated Ca²⁺ channels (VACC) and neuronal nicotinic receptors. A very high concentration of MRZ 2/579 (1 mM) partially blocked non-differentiated VACCs in cultured hippocampal neurones in a voltage-dependent manner (data not shown). The concentration

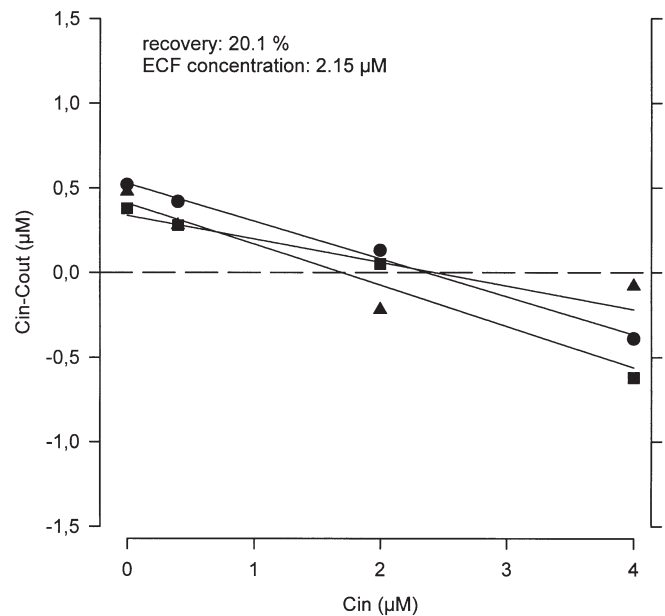


Fig. 4 Graph presenting data from an *in vivo* recovery experiment based on the Zero-Net-Flux method (ZNF). Microdialysis probes placed in the striatum were perfused with increasing concentrations of MRZ 2/579 and the content in outgoing aCSF was assessed. When ECF and ingoing concentrations were equal, no change in the outgoing concentration occurred. Results are examples of three rats perfused with four concentrations of MRZ 2/579

dependency of MRZ 2/579 at VACCs was determined against maximal inward currents following 100-ms steps from -70 mV to -10 mV (Fig. 2). MRZ 2/579 did indeed block these currents, but with very low potency ($IC_{50}=340.0 \pm 14.3$ μ M). High concentrations of memantine also block neuronal nicotinic receptors with an IC_{50} of 12.3 μ M (Parsons et al. 1998). Under similar conditions (Ach 1 mM in the presence of 1 μ M atropine), MRZ 2/579 was even weaker as a neuronal nicotinic receptor antagonist producing moderate block of peak responses ($72.3 \pm 3\%$ of control) at a concentration of 10 μ M and no effect on steady-state responses (Fig. 3). MRZ 2/579 was also tested for antagonistic effects at AMPA and GABA_A receptors. As previously reported for memantine (Chen et al. 1992; Parsons et al. 1993), MRZ 2/579 (10–100 μ M) had little or no effect on inward currents to GABA (10 μ M) or AMPA (100 μ M) (Fig. 3).

In vivo experiments

In the ZNF experiment an *in vivo* recovery of $20.1 \pm 3.4\%$ was calculated. (Fig. 4) This was comparable to the *in vitro* recovery of MRZ 2/579 ($17.7 \pm 1.6\%$, water recovery method, 37°C , non-stirred). All subsequent microdialysate concentrations were corrected for the *in vivo* recovery of MRZ 2/579.

Following 7 days of s.c. infusion (40 mg/kg day) MRZ 2/579 reached a concentration in the brain ECF of 0.36 μ g/ml (2.15 μ M; Table 1). This was comparable to its

Table 1 Distribution of MRZ 2/579 between plasma (total), CSF, whole brain tissue, and brain ECF after chronic administration of 40 mg/kg day for 7 days (means \pm SEM, $n=4$). The difference between plasma, CSF and brain ECF was not significant (Student's *t*-test)

Plasma ($\mu\text{g/ml}$)	CSF ($\mu\text{g/ml}$)	Brain tissue ($\mu\text{g/g}$)	Brain ECF ($\mu\text{g/ml}$)
0.73 \pm 0.16 (4.3 \pm 0.94 μM)	0.36 \pm 0.08 (2.16 \pm 0.44 μM)	21.2 \pm 6.6	0.36 \pm 0.04 (2.15 \pm 0.2 μM)

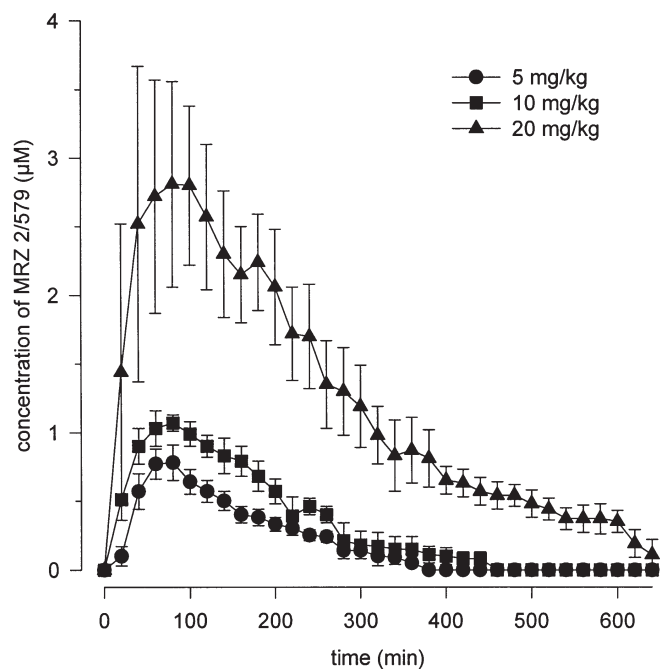


Fig. 5 Free brain ECF concentration of MRZ 2/579 following a single bolus administration of MRZ 2/579 (5, 10 or 20 mg/kg i.p.). Dialysate concentrations are corrected for in vivo recovery (means \pm SEM, $n=3-5$)

Table 2 Pharmacokinetic parameters ($T_{1/2}$, AUC) of MRZ 2/579 in the brain ECF as measured with brain microdialysis corrected for in vivo recovery. For plasma and brain tissue (homogenates) rats were sacrificed at various intervals after injection and the tissue collected. Rats were injected with 5, 10 or 20 mg/kg i.p. (means \pm SEM, $n=3-5$)

Tissue	Parameter	Dose of MRZ 2/579 (mg/kg)		
		5	10	20
Plasma	$T_{1/2}$ (h)	1.63	2.65	2.63
	AUC ($\mu\text{g/mlh}$)	1.26	2.93	6.03
Brain tissue	$T_{1/2}$ (h)	6.79	1.99	1.99
	AUC ($\mu\text{g/mlh}$)	47.8	77.1	206.8
ECF	$T_{1/2}$ (h)	1.95 \pm 0.198	2.23 \pm 0.36	2.94 \pm 0.064
	AUC ($\mu\text{g/mlh}$)	0.32 \pm 0.054	0.54 \pm 0.08	2.06 \pm 0.487

concentration in the CSF (0.36 $\mu\text{g/ml}$, 2.16 μM). Plasma levels (0.73 $\mu\text{g/ml}$, 4.33 μM) were almost twofold higher than brain ECF concentrations. Ultrafiltration experiments revealed that 22.2% of MRZ 2/569 in plasma was bound to protein, meaning that brain ECF to free plasma ratio was 0.634. The whole brain tissue concentration (21.2 $\mu\text{g/g}$ tissue) was however considerably higher than brain ECF, CSF and plasma levels. This constitutes a 58-fold accumulation in brain tissue compared to brain ECF.

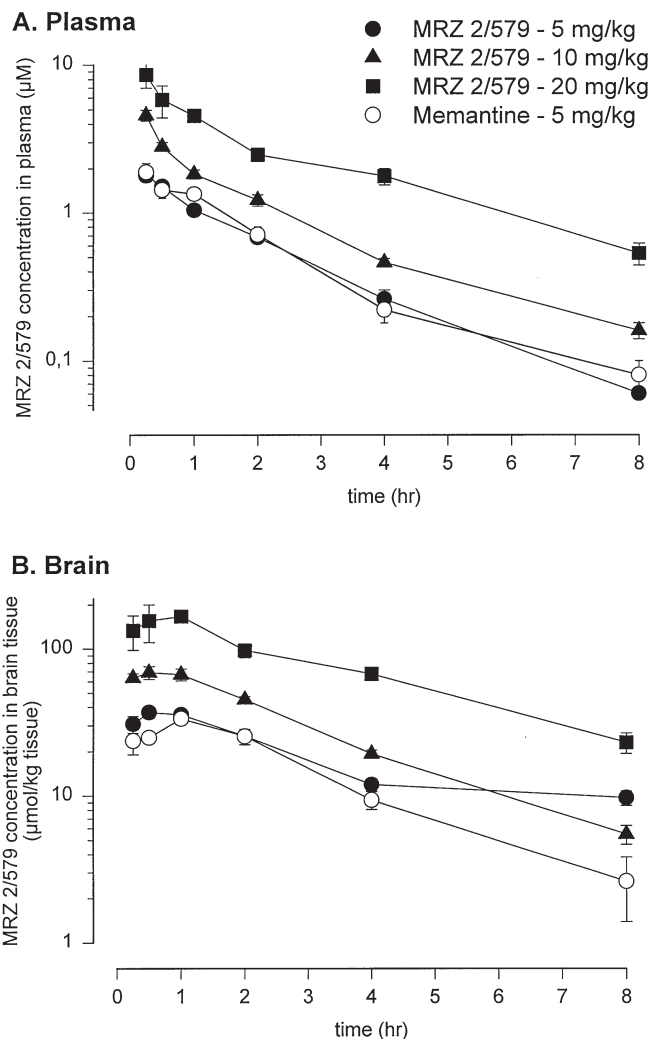


Fig. 6 **A** Plasma and **B** brain tissue concentration-time profile after administration of MRZ 2/579. The animals were sacrificed at various time intervals after administration of MRZ 2/579 (5, 10 and 20 mg/kg i.p.) and a blood sample was collected by heart puncture and the brain removed. For comparison, data on memantine (5 mg/kg i.p.) are shown (means \pm SEM, $n=5$)

After an initial distribution phase, the peak concentration of MRZ 2/579 (0.70, 0.96 and 2.53 μM after acute i.p. administration of 5, 10 and 20 mg/kg, respectively) was reached in the brain after 80–100 min (Fig. 5). This was followed by an elimination phase characterized by an elimination half-life of 2–3 h depending on the dose (Table 2; Fig. 6). It should be noted that the peak ECF concentrations reached were not linearly related to the dose administered, indicating that MRZ 2/579 elimination may be limited by a saturable process. This is clearly seen

comparing the AUC values (Table 2; Fig. 6). Similarly, the plasma and brain homogenates AUCs were not linearly related to the dose, indicating that this is not specific for the brain compartment. Generally, MRZ 2/579 had a similar half-life to memantine since at 5 mg/kg i.p. their respective half-lives were 1.63 h and 1.95 h (not shown for memantine).

Discussion

The present study demonstrates that after application of MRZ 2/579 at doses previously shown to be active in animal models, free brain ECF concentrations are close to the potency of this agent as an NMDA receptor antagonist. These estimates were obtained using brain microdialysis with in vivo recovery – ZNF – which is believed to be the best method for this kind of study. MRZ 2/579 is at least 100-fold selective for NMDA vs. GABA_A, AMPA and VACC and more than tenfold selective versus neuronal nicotinic receptors (type 1A responses).

Following chronic s.c. infusion of MRZ 2/579 (40 mg/kg day for 7 days) brain ECF (2.15 μ M) and CSF levels (2.16 μ M) were twofold lower than free plasma levels (4.3 μ M). MRZ 2/579 showed pronounced accumulation in brain tissue compared to free plasma (28-fold) and ECF (58-fold). This would indicate that as far as its brain distribution properties are concerned, MRZ 2/579 is a 'typical' uncompetitive NMDA receptor antagonist, since accumulation in brain tissue relative to CSF and plasma/serum has been shown for a number of related compounds. For the aminoadamantanes memantine and amantadine this effect probably reflects accumulation into the lysosomal compartment (Honegger et al. 1993). This has been shown to be a dynamic process in vitro, i.e. the accumulated aminoadamantanes are released upon removal from the culture medium. In patients, the aminoadamantane concentration in brain tissue has been found to be 20–30 times higher than in serum and CSF (Pantev et al. 1993; Kornhuber and Quack 1995; Danysz et al. 1997). The same has been observed in experimental animals (Miształ et al. 1996; Hesselink et al. 1999). In this instance free brain ECF concentrations were determined to be comparable to free plasma and CSF concentrations (Hesselink et al. 1999). Other uncompetitive NMDA receptor antagonists known to accumulate in brain tissue are: (+)MK-801, ketamine, dextrophan and dextromethorphan (Vezzani et al. 1989; Schwartz and Wasterlain 1993; Hartvig et al. 1995). For (+)MK-801, Schwartz and Wasterlain (1993) also reported that free plasma and free brain ECF concentrations were comparable despite pronounced accumulation in brain tissue. The mechanism by which these compounds accumulate in brain tissue is not fully elucidated but seems to be determined by lipophilicity (log P) and acidotropism (pK_a; Kornhuber et al. 1995). The latter feature is responsible for the accumulation in the lysosomal compartment which is acidic (Honegger et al. 1993). Apart from NMDA receptor antagonists, such accumulation in the brain tissue has been described for

many drugs such as antidepressants, neuroleptics, tacrine etc. (Kornhuber et al. 1995; McNally et al. 1996).

In the patch clamp experiments in cultured hippocampal neurones, the observed blockade of NMDA receptors by MRZ 2/579 was typical for a moderate-affinity uncompetitive NMDA receptor antagonist. Steady-state inward current responses of cultured hippocampal neurones to NMDA were antagonized by MRZ 2/579 with an IC₅₀ of 1.11±0.09 μ M at –70 mV. The peak concentrations in brain ECF after administration of 5, 10 or 20 mg/kg i.p. (0.70, 0.96 and 2.53 μ M, respectively) are close to or slightly greater than this IC₅₀ value. This is in good agreement with the behavioural studies performed with this compound. Its antiparkinsonian-like activity, i.e. antagonism of haloperidol-induced catalepsy, is observed starting at 10 mg/kg (Karcz-Kubicha et al. 1999). In a study by Wenk and co-workers (1998), MRZ 2/579 at 10 mg/kg provided full protection against NMDA-induced neurotoxicity in rats, whereas at 5 mg/kg partial protection was observed. In Wistar rats, a dose of 5 mg/kg inhibited both the acquisition and expression of morphine-induced conditioned place preference (Popik et al. 1998). These studies could indicate that if no major strain differences in pharmacokinetics exist, only modest NMDA receptor blockade by MRZ 2/579 would be required to exert some functional effects.

The therapeutic significance of lysosomal accumulation of MRZ 2/579, memantine and related compounds is uncertain. However, the high intracellular concentrations do not seem to be directly involved in NMDA receptor antagonism. Thus, taken together with previous data from behavioural studies it seems that the "therapeutic" effects in vivo are mediated at free ECF concentrations in line with the in vitro potency of open NMDA receptor channel blockade from the extracellular compartment. In contrast to Mg²⁺, high concentrations of memantine (30 μ M) do not block open NMDA receptor channels from the intracellular compartment (Spielmans, unpublished). Much higher concentrations are required to affect other Ca²⁺-permeable channels or other ligand-gated ionotropic receptors.

In conclusion, the concentration of MRZ 2/579 in the brain ECF that is reached after administration of behaviourally active doses is within the range of its IC₅₀ at the NMDA receptor in spite of up to 58-fold accumulation in the brain tissue. At this concentration a significant degree of selectivity for NMDA receptors can be expected.

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