Novel Systemically Active Antagonists of the Glycine Site of the N-Methyl-d-aspartate Receptor: Electrophysiological, Biochemical and Behavioral Characterization

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ABSTRACT

A series of novel tricyclic pyrido-phthalazine-dione derivatives was tested for antagonistic effects at the strychnine-insensitive modulatory site of the N-methyl-d-aspartate (NMDA) receptor (glycineB). All compounds displaced [3H]MDL-105,519 binding to rat cortical membranes with IC50 values of between 90 nM and 3.8 μM. In patch-clamp experiments, steady-state inward current responses of cultured hippocampal neurons to NMDA (200 μM, glycine 1 μM) were antagonized by these same compounds with IC50 values of 0.14 to 13.8 μM. The antagonism observed was typical for glycineB antagonists, i.e., they induced desensitization and their effects were not use or voltage dependent. Moreover, increasing concentrations of glycine were able to decrease their apparent potency. Much higher concentrations (>100 μM) were required to antagonize α-aminoo-3-hydroxy-5-methyl-4-isoxazolepropionic acid-induced currents. They were potent, systemically active NMDA receptor antagonists in vivo against responses of single neurons in the rat spinal cord to microelectrophoretic application of NMDA with ID50 values in the low milligram per kilogram i.v. range. They also inhibited pentyleneetrazol-, NMDA- and maximal electroshock-induced convulsions in mice with ED50 values ranging from 8 to 100 mg/kg i.p. The duration of anticonvulsive action was rather short but was prolonged by the organic acid transport inhibitor probenecid (200 mg/kg). The agents tested represent a novel class of systemically active glycineB antagonists with greatly improved bioavailability.

Glutamate is probably the major excitatory transmitter in the CNS but is also likely to be involved in numerous pathological and excitotoxic processes (see Danysz et al., 1995). It is therefore not surprising that there is a great deal of interest in the development of glutamate antagonists for therapeutic use (see Meldrum, 1985; Lipton and Rosenberg, 1994; Muir and Lees, 1995; Danysz et al., 1995). Glutamate activates three major types of ionotropic receptor, namely AMPA, kainate and NMDA and several types of metabotropic receptors.

Antagonism of NMDA receptors may potentially have a wide range of therapeutic applications ranging from acute neurodegeneration (e.g., stroke), chronic neurodegeneration (e.g., Parkinson’s disease, Alzheimer’s disease, Huntington’s disease) to symptomatic treatment (e.g., Parkinson’s disease, drug dependence, depression, anxiety, chronic pain, etc.). Functional modulation of NMDA receptors can be achieved through actions at different recognition sites such as: the primary transmitter site (competitive), the phencyclidine site and the strychnine-insensitive, co-agonistic glycine site (glycineB, Danysz et al., 1987; Johnson and Ascher, 1987; Wong et al., 1987; Kleckner and Dingle-dine, 1988; Fadda et al., 1988).

Although several uncompetitive and competitive NMDA receptor antagonists are already used clinically or are at an advanced stage of development (Turski, 1990; Lipton and Rosenberg, 1994; Danysz et al., 1995) less is known about the therapeutic potential of full antagonists acting at the glycineB site (Carter, 1992; Kemp and Leeson, 1993; Leeson and Iverson, 1994; Kulagowski and Leeson, 1995). Initial preclinical evidence, which suggested that a different, perhaps more

ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, central nervous system; DH, dorsal horn; GABA, γ-aminobutyric acid; GlycineB, strychnine-insensitive, co-agonistic glycine site of the NMDA receptor; MES, maximal electroshock; NMDA, N-methyl-d-aspartate; PTZ, pentyleneetrazol; TI, therapeutic index; 5,7-DCKA, 5,7-dichlorokynurenic acid; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; ACPC, 1-aminocyclopropanecarboxylic acid.
promising therapeutic profile might be expected from glycine\textsubscript{B} antagonists, was obtained either with local intracerebroventricular administration of full glycine\textsubscript{B} antagonists with poor pharmacokinetic properties or systemic administration of partial agonists (e.g., Moroni et al., 1992; Bubser et al., 1992; Baran et al., 1994; Pellegrini-Giampietro et al., 1994; Salituro et al., 1994). In such studies glycine\textsubscript{B} antagonists have lacked many of the side effects classically associated with NMDA receptor blockade, such as: 1) lack of neurodegenerative changes in the cingulate/retrosplenial cortex even after high doses (Chen et al., 1993; Haggerty et al., 1993; Hargreaves et al., 1993; Berger et al., 1994); 2) lack of psychotomimetic-like effects (Koek and Colpaert, 1990; Danysz et al., 1994; L"oscher et al., 1994; Tortella and Hill, 1996); 3) lack of learning-impairing effects at anticonvulsive doses (Chiamulera et al., 1990; Murata and Kawasaki, 1993; Faiman et al., 1994).

However, some full glycine\textsubscript{B} antagonists with improved, but by no means optimal pharmacokinetic properties (Baron et al., 1992; Carling et al., 1993; Rowley et al., 1993; Woodward et al., 1995) were also reported to have good therapeutic indices after systemic administration in models of hyperalgesia (Vaccarino et al., 1993; Millan and Seguin, 1994; Laird et al., 1996), as anxiolytics (Kehne et al., 1995), as possible anti-psychotomimetics (Bristow et al., 1995, 1996), as neuroprotective agents in models of focal ischemia (Warner et al., 1995) and trauma (Tsuchida and Bullock, 1995) as antiepileptics, even in models of partial complex seizures (McCabe et al., 1993; Chapman et al., 1995; Smith et al., 1994) and in blocking spreading depression (Obrenovitch and Zilka, 1996). These studies seem to confirm the promising therapeutic profile of systemically active glycine\textsubscript{B} antagonists.

Merz has developed a series of tricyclic “pyrido-phthalazine-diones” which are also moderately potent glycine\textsubscript{B} antagonists in vitro but show a much better in vivo systemic availability and/or penetration of the blood-brain barrier than most glycine\textsubscript{B} receptor antagonists reported to date.

Methods

In vitro Receptor Binding Studies

Membrane preparation and protein determination. Tissue preparation was performed according to Foster and Wong (1987). Male Sprague-Dawley rats (200–250 g) were decapitated and their brains were removed rapidly. The cortex was dissected and homogenized in 20 volumes of ice-cold 0.32 M sucrose with a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 \( \times g \) for 10 min. The pellet was discarded and the supernatant was centrifuged at 20,000 \( \times g \) for 20 min. The resulting pellet was resuspended in 20 volumes of distilled water and centrifuged for 20 min at 8000 \( \times g \). Then the supernatant and the buffy coat were centrifuged three times (48,000 \( \times g \) for 20 min) in the presence of 50 mM Tris-HCl, pH 8.0. All centrifugation steps were carried out at 4°C. After resuspension in 5 volumes of 50 mM Tris-HCl, pH 8.0, the membrane suspension was frozen rapidly at \(-80°C\). On the day of assay the membranes were thawed and washed four times by resuspension in 50 mM Tris-HCl, pH 8.0, and centrifugation at 48,000 \( \times g \) for 20 min. The final pellet was suspended in assay buffer. The amount of protein in the final membrane preparation was determined according to the method of Lowry et al. (1951) with some modifications (Hartree, 1971). The final protein concentration used for our studies was between 250 and 500 \( \mu g/mL \).

In initial experiments, stock solutions (10 mM) were made in distilled water or dimethyl sulfoxide. In later experiments with [\(^3\)H]5,7-DCKA and [\(^3\)H]MDL-105,519 most stock solutions were made in 10% saturated Tris buffer (pH 9.5) to improve solubility further. Serial dilutions of these stock solutions were then made in distilled water before addition of 50 \( \mu l \) aliquots to the assay tubes (total volume, 500 \( \mu l \)).

[\(^3\)H]5,7-DCKA binding assay. Incubations were performed according to the methods modified from previous groups (Yoneda et al., 1993). Membranes were suspended and incubated in 50 mM Tris-HCl, pH 8.0, for 45 min at 4°C with a fixed [\(^3\)H]5,7-DCKA concentration of 10 nM. Nonspecific binding was defined by the addition of unlabeled glycine at 1 mM.

[\(^3\)H]MDL-105,519 binding assay. MDL-105,519 is a novel, selective high-affinity antagonist at the glycine\textsubscript{B} site and was recently introduced as a commercially available radioligand for binding studies at this site (Baron et al., 1996, 1997; Hofner and Wanner, 1997). Experiments with [\(^3\)H]MDL-105,519 were performed as with [\(^3\)H]5,7-DCKA, except that a fixed [\(^3\)H]MDL-105,519 concentration of 2 nM was used.

[\(^3\)H]Glycine binding assay. [\(^3\)H]Glycine-binding assays were performed according to Kessler et al. (1989). Membranes were suspended and incubated in 50 mM Tris-acetate, pH 7.4. Compounds were incubated with 20 nM [\(^3\)H]Glycine for 30 min at 4°C in the presence of 100 \( \mu M \) strychnine. Nonspecific binding was defined by the addition of unlabeled 5,7-DCKA (10 \( \mu M \)).

Incubations were terminated using a Millipore filter system. The samples, all in duplicate, were rinsed three times with 2.5 ml ice-cold assay buffer over glass-fiber filters obtained from Schleicher and Schuell (Keene, NH) under a constant vacuum. For the [\(^3\)H]Glycine assay, 10 mM MgSO\(_4\) was added to the stop buffer. Filtration was performed as rapidly as possible. After separation and rinse the filters were placed into scintillation liquid (5 ml; Ultima Gold) and radioactivity retained on the filters was determined with a conventional liquid scintillation counter (Liquid Scintillation Analyser, Hewlett Packard, Palo Alto, CA).

Patch Clamp

Hippocampi were obtained from rat embryos (E20 to E21) and were then transferred to calcium- and magnesium-free Hanks’ buffered salt solution (Gibco, Eggenstein, Germany) on ice. Cells were mechanically dissociated in 0.05% DNase/0.3% ovomucoid (Sigma, Deisenhofen, Germany) after an 8-min preincubation with 0.66% trypsin/0.1% DNase (Sigma, Deisenhofen, Germany). The dissociated cells were then centrifuged at 18 \( \times g \) for 10 min, resuspended in minimum essential medium (Gibco, Eggenstein, Germany) and plated at a density of 150,000 cells cm\(^{-2}\) onto poly-\(\beta\)-lysine (Sigma)/laminin (Gibco) poly-\(\ell\)-lysine (Sigma)-precoated plastic Petri dishes (Falcon, Heidelberg, Germany). The cells were nourished with NaHCO\(_3\)/HEPES-buffered minimum essential medium supplemented with 5% fetal calf serum and 5% horse serum (Gibco, Egg- enstein, Germany) and incubated at 37°C with 5% CO\(_2\) at 95% humidity. The medium was exchanged completely after inhibition of further glial mitosis with cytosine-\(\beta\)-d-arabinofuranoside (5 mM Sigma) after about 5 days in vitro. Thereafter the medium was exchanged partially twice weekly.

Patch-clamp recordings were made from these cultured neurons, after 12 to 15 days in vitro, with polished glass electrodes (3–5 meq/mH) 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– to 20-ms exchange times). The contents of the intracellular solution were as follows (mM): CsCl, 120; triethanolamine-Cl, 20; ethyleneglycol-bis-(\(\beta\)-aminoethyl ether)-N,N,N\textsubscript{3},N\textsubscript{4}-tetraacetic acid, 10; MgCl\(_2\), 1; CaCl\(_2\), 0.2; glucose, 10; ATP, 2; cAMP, 0.25; pH was adjusted to 7.3 with CaOH or HCl. The extracellular solutions had the following basic composition (mM): NaCl, 140; KCl, 3; CaCl\(_2\), 0.2; glucose, 10; HEPES, 10; sucrose, 4.5; tetrodotoxin (3 \( \times 10^{-4}\)). For most experiments glycine (1 \( \mu M \)) was present in all solutions, a concentration sufficient to cause approxi-
mately 80% activation of glycine\textsubscript{B} receptors. Experiments to test the glycine dependence of the Merz glycine\textsubscript{B} antagonists were performed in the continuous presence of increasing concentrations of glycine (1–10 \mu M). Only results from stable cells were accepted for inclusion in the final analysis, i.e., after recovery of responses to NMDA by at least 75% of their depression by the antagonists tested.

**Microelectrophoretic Application of NMDA and AMPA to Spinal Neurons in Vivo**

Details of the experimental procedure have been described elsewhere (Cumberbatch et al., 1995). Male Wistar rats (290–350 g) were anesthetized with halothane, and tracheal, carotid and jugular can- nulae were inserted. A laminectomy (T10–L2) was performed, and the spinal cord was cut at T10–T11. Anesthesia was switched to anesthetized with halothane, and tracheal, carotid and jugular cannulae were inserted. A laminectomy (T10–L2) was performed, and the spinal cord was cut at T10–T11. Anesthesia was switched to and maintained with \alpha-chloralose (50 mg/kg i.v., then 10–15 mg/kg/hr).

Arterial blood pressure was monitored continuously and systolic pressure remained above 100 mm Hg. Core temperature was maintained close to 37°C.

Extracellular recordings of single DH neuron action potentials were made by multibarrel glass electrodes containing 3.5 M NaCl (recording barrel), sodium salts of NMDA (100 mM in 100 mM NaCl) and AMPA (10 mM in 200 mM NaCl), both at pH 7.5 to 8.0, and 150 mM NaCl for automated current balancing. Amino acids were ejected in regular cycles (40–s ejection period, 30–40 s interstimulus interval) with 60-s intervals between cycles. Drugs were dissolved in an aqueous solvent (0.606 g Tris; 5.0 g glucose; 0.5 g Tween 80; 95 ml water) at pH 8.5 to 9.0 (MRZ 2/502 and 2/516) or in 0.9% saline (MRZ 2/570, 2/571, 2/576 and 2/577). Typical drug concentrations were 3 to 5 mg/ml. Drugs were given intravenously in a cumulative dose-doubling regime when the variability of the control amino acid responses did not exceed 10%. Drug effects were expressed as percentages of the mean of last three predrug responses. Spike counts were compensated for ongoing activity. Calculations of ED\textsubscript{50} values were performed on individual cells with commercially available software (GraFit, Erithacus Software Ltd, Staines, Middlesex, England). Statistical analysis was by the Mann-Whitney test on original spike responses did not exceed 10%. Drug effects were expressed as percentages of the mean of last three predrug responses. Spike counts were compensated for ongoing activity. Calculations of ED\textsubscript{50} values were performed on individual cells with commercially available software (GraFit, Erithacus Software Ltd, Staines, Middlesex, England). Statistical analysis was by the Mann-Whitney test on original spike count data.

**Anticonvulsive Activity**

Male albino Swiss mice (19–21 g) housed 10 to 15 per cage were used for the NMDA lethality test (Leander et al., 1988). For PTZ-induced convulsions male albino Swiss mice (25–34 g) housed 40 per cage were used, whereas in the MES and motor impairment tests NMRI female mice (18–28 g) housed 5 per cage were used. All animals were kept with water and food ad libitum under a 12-h light-dark cycle (light on at 6 A.M.) and at a controlled temperature (20 ± 0.5°C). All experiments were performed between 10 A.M. and 5 P.M. Tested agents were usually injected i.p. 15 min before the induction of convulsions. MRZ 2/502 was dissolved in saline with NaOH added. The choline salts of Merz glycine\textsubscript{B} antagonists were dissolved in distilled water. Most other agents were dissolved in the following solution: 0.606 g Tris; 5.0 g glucose; 0.5 g Tween 80; 95 ml water. All experiments were performed strictly according to the animal rights commission allowance F 77–51 (Hessen).

In the test of NMDA-induced convulsions in mice, a dose-response relationship for NMDA was first performed to determine the ED\textsubscript{50} dose which was then used for testing antagonistic properties. After injection of the ED\textsubscript{50} dose of NMDA the animals were placed in a small cage and observed for 20 min. Death preceded by clonic action (traction reflex) and motor coordination (rotarod). For the traction reflex test mice were placed with their forepaws on a horizontal rod and were required to place all four paws on the wire within 10 sec. To test ataxia (motor coordination) mice were placed on rotarod (5 rpm) and were required to remain on the rod for 1 min. Only mice not achieving the criteria in all three repetitions of each test were considered to exhibit myorelaxation or ataxia, respectively. These tests were followed by MES (100 Hz, 0.5-s shock duration, 50-mA shock intensity, 0.9-ms impulse duration, Ugo Basile, Comerio, Italy) applied through corneal electrodes. The presence of tonic convulsions was scored (tonic extension of hind paws with minimum angle to the body of 90°). The aim was to obtain ED\textsubscript{50} values for all parameters scored (anticonvulsive activity and motor side effects) with use of the Litchfield Wilcoxon test for quantal dose responses. Division of the ED\textsubscript{50} for side effects (ataxia or myorelaxation) by the ED\textsubscript{50} for antagonism of electroshock convulsions was used as a TI.

**Chemicals**

The following agents were used: ACPC, S-AMPA, 7-chlorotrihydroxykynurenine acid, 5,7-dichlorotrihydroxykynurenine acid, d-cycloserine, 5,7-DCKA, 3-amino-1-hydroxypropyridolin-2-one ((R)-HA-966), kynurenic acid, trans-2-carboxy-5,7-dichloro-4-phenylaminobenzylamino-1,2,3,4-tetrahydroquinoline (L-689,560), 7-chloro-3-(cylopropylcarbonyl)-4-hydroxy-2(1H)-quinoline (L-701,252), 3-(3-hydroxyphenyl)prop-2-ynyl-7-chloro-4-hydroxy-2-(1H)-quinoline-3-carboxylate (L-701,273), 7-chloro-4-hydroxy-3-(4-phenoxyphenyl)-2H-quinoline (L-701,324), d-serine, l-serine (all Tocris Cookson Ltd, Bristol, U.K.); NMDA, glycine, tetrodotoxin, PTZ, warfarin, probenecid, salts and buffers (all Sigma); all pyrido-phthalazine-dione derivatives (Institute for Organic Synthesis, Riga, Latvia). The basic structure of these derivatives is given in figure 1. The following radioligands were used: [3H]15,7-DCKA (56.6 Ci/mmol) and [3H]glycine (48.4 Ci/mmol) (Dupont NEN, Cologne, Germany); [3H]MDL-105,519 ([3H](Z)-2-(phenyl)-3-(4,6-dichloroindol-3-yl-2-carboxylic acid) propenoic acid, 74.0 Ci/mmol) (Amersham, Braunschweig, Germany).

**Results**

**In Vitro Receptor Binding Studies**

The tricyclic pyrido-phthalazine-diones tested displaced [3H]MDL-105,519 binding to rat cortical membranes with IC\textsubscript{50} values of between 90 nM and 3.9 \mu M (see table 1). Standard glycine\textsubscript{B} antagonists, partial agonists and full agonists also showed their expected potencies in this assay (see table 1). Nonspecific binding determined with 1 mM glycine was only 10 to 15% and all compounds tested displaced binding to nonspecific levels with Hill coefficients very close to unity (fig. 2).

In contrast, although potencies were roughly similar in the [3H]MDL-105,519 binding to rat cortical membranes with IC\textsubscript{50} values of between 90 nM and 3.9 \mu M (see table 1). Standard glycine\textsubscript{B} antagonists, partial agonists and full agonists also showed their expected potencies in this assay (see table 1). Nonspecific binding determined with 1 mM glycine was only 10 to 15% and all compounds tested displaced binding to nonspecific levels with Hill coefficients very close to unity (fig. 2).

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![Fig. 1. Basic structure of the pyrido-phthalazine-diones.](image-url)
remains unclear and confounded the interpretation of the data and resulted, in many cases, in Hill coefficients as low as 0.5 and associated high variability in the data (see also Baron et al., 1991; Yoneda et al., 1993). As such the results are not presented. [3H]MDL-105,519 binding therefore seems to be the more suitable, commercially available radioligand for determining binding to the glycineB site.

**Patch Clamp**

Steady-state inward current responses of cultured hippocampal neurons to NMDA (200 μM with 1 μM glycine) were antagonized by these tricyclic pyrido-phthalazine-diones with IC_{50} values of 0.14 to 13.8 μM. The relative potencies of standard glycineB antagonists, partial agonists and full agonists were similar to those reported in the literature and correlated well with [3H]MDL-105,519 binding (Pearson Product Moment Correlation Coefficient = 0.941, P < .0001). The antagonism observed with most compounds was typical for glycineB full antagonists, i.e., they induced glycine-sensitive desensitization (fig. 3). The degree of this desensitization, however, seemed to depend largely on the potency of antagonist tested: 5,7-DCKA and 7-chlorokynurenic acid were 10 times more potent against steady-state currents than against peak currents, whereas L-689,560 and L-701,324 were almost equieffective against these two components (table 2). In agreement with previous reports, partial agonists were equipotent against both components despite showing relatively low potency (Kemp and Priestley, 1991). The NMDA receptor antagonism by Merz tricyclic pyrido-phthalazine-diones was mediated at the glycineB site as evidenced by the parallel shift in the concentration-response curves in the presence of increasing glycine concentrations.

**TABLE 1**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC_{50} (nM)</th>
<th>S.E.</th>
<th>Hill coefficient</th>
<th>S.E.</th>
<th>n</th>
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<td>MRZ 2/499  Acid</td>
<td>2991.1</td>
<td>280.1</td>
<td>1.11</td>
<td>0.05</td>
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<td>MRZ 2/577  Choline salt</td>
<td>3857.6</td>
<td>247.2</td>
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<td>0.04</td>
<td>4</td>
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<td>MRZ 2/502  Acid</td>
<td>90.9</td>
<td>0.85</td>
<td>1.03</td>
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<td>14.2</td>
<td>0.93</td>
<td>0.02</td>
<td>4</td>
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<tr>
<td>MRZ 2/514  Acid</td>
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<td>0.91</td>
<td>1.00</td>
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<td>MRZ 2/570  Choline salt</td>
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<td>16.1</td>
<td>0.95</td>
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<td>0.89</td>
<td>0.98</td>
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<td>0.38</td>
<td>1.13</td>
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<td>DCKA⁶</td>
<td>80.1</td>
<td>17.5</td>
<td>0.90</td>
<td>0.04</td>
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<tr>
<td>5,7-DIC-thio-KA</td>
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<td>4.9</td>
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<tr>
<td>7-Cl-thio-KA</td>
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<td>11.4</td>
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<td>0.05</td>
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<tr>
<td>Kynurenic acid</td>
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<tr>
<td>Glycine</td>
<td>304.0</td>
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<td>0.84</td>
<td>0.01</td>
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<tr>
<td>(+)-HA-966</td>
<td>1810.0</td>
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</table>

* Estimation of IC_{50} values and curve fitting were made according to the four-parameter logistic equation (Excel, Microsoft Software). Data are presented as means ± S.E. from separate experiments.

⁶ 5,7-Dichlorokynurenic acid.

⁷ 1-Aminocyclopropanecarboxylic acid.

Fig. 2. Representative experiment showing the concentration-dependent displacement of [3H]MDL 105,519 binding to cortical membranes by glycineB antagonists. Membranes were incubated with [3H]MDL 105,519 (2 nM) in 50 mM Tris-HCl, pH 8.0, for 45 min at 4°C. Nonspecific binding (13.2 ± 0.2%) was defined by the addition of unlabeled glycine (1 mM). The effects of each concentration of displacer were assessed in duplicate and plotted as % specific binding against concentration. Estimation of IC_{50} values and curve fitting were made according to the four-parameter logistic equation (GraFit, Erithacus Software).

DCKA
Kynurenic Acid
Glycine
D-Serine

![Diagram](https://example.com/diagram.png)
Thus the $K_b$ values of MRZ 2/502 as assessed according to the Cheng-Prussoff relationship were similar in 1, 3 and 10 $\mu$M glycine (79.9 ± 18.3, 124.4 ± 29.3 and 118.2 ± 24.8 nM corresponding to $I_{C50}$ values of 0.28 ± 0.03, 1.06 ± 0.09 and 3.08 ± 0.14 $\mu$M, respectively). Furthermore, the effects of MRZ 2/502 were not voltage-dependent (fig. 4). Choline derivatives had potencies similar to the free acids in vitro (table 2).

In contrast, the three of these glycine$_B$ antagonists tested were only very weak antagonists of inward currents to AMPA.
MRZ 2/502, 2/514 and 2/516 had IC$_{50}$ values against peak AMPA-induced currents of 25.1 ± 2.0, 72.7 ± 6.4 and 17.6 ± 1.5 μM, respectively, but were essentially inactive against steady-state currents that had IC$_{50}$ values.

This profile of action, although very weak, is typical for competitive AMPA receptor antagonists which preferentially block the peak nondesensitized, low-affinity state of the AMPA receptor (see Parsons et al., 1994).

(100 μM). MRZ 2/502, 2/514 and 2/516 had IC$_{50}$ values against peak AMPA-induced currents of 25.1 ± 2.0, 72.7 ± 4.7 and 17.6 ± 1.5 μM, respectively, but were essentially inactive against steady-state currents that had IC$_{50}$ values > 100 μM (fig. 5). This profile of action, although very weak, is typical for competitive AMPA receptor antagonists which preferentially block the peak nondesensitized, low-affinity state of the AMPA receptor (see Parsons et al., 1994).

**Fig. 4.** (A) Glycine counteracts the antagonistic effects of MRZ 2/502 on NMDA-induced currents. NMDA (200 μM) was applied for 2.5 s every 30 s at −70 mV in the continuous presence of glycine (1, 3 and 10 μM). Pooled steady-state responses were quantified as in figure 3 and plotted against antagonist concentration (4–8 per concentration). The four-parameter logistic equation was used to fit the data (solid curves) and to calculate the IC$_{50}$ values for MRZ 2/502 (1 μM glycine, 0.28 ± 0.03 μM, Hill coefficient 0.96 ± 0.05; 3 μM glycine, 1.06 ± 0.08 μM, Hill coefficient 1.09 ± 0.06; 10 μM glycine, 3.08 ± 0.14 μM, Hill coefficient 1.23 ± 0.06). The K$_{B}$ values of MRZ 2/502 as assessed according to the Cheng-Prussoff relationship were similar in 1, 3 and 10 μM glycine (79.9 ± 18.3, 124.4 ± 29.3 and 118.2 ± 24.8 nM, respectively). (B) Voltage independence of the blockade of NMDA receptors by MRZ 2/502. NMDA (200 μM) was applied for 2.5 s every 30 s in the continuous presence of glycine (1 μM) at various membrane potentials. Peak and plateau (steady-state) NMDA current responses in the absence and presence of MRZ 2/502 have been plotted as means against membrane potential (n = 2). The upper left insert shows original data for the i.v. curve in the presence of MRZ 2/502 (1 μM).

**Fig. 5.** Higher concentrations of MRZ 2/502 also antagonize AMPA-induced inward currents in cultured hippocampal neurons. (A) AMPA (100 μM) was applied for 1 s every 15 s at −70 mV. The left and right profiles show control and recovery responses, respectively. The middle three profiles show equilibrium responses in the continuous presence of 10, 30 and 100 μM MRZ 2/502, respectively. (B) Pooled responses were quantified as peak and steady-state (plateau) currents after subtraction of any leak current and plotted, after normalization to control, as means ± S.E. against MRZ 2/502 concentration. At least six cells were tested at each concentration. The four-parameter logistic equation was used to fit the data (solid curves) and to calculate the IC$_{50}$ values of MRZ 2/502: Peak IC$_{50}$ = 25.1 ± 2.0 μM (Hill coefficient = 0.72 ± 0.04); steady-state IC$_{50}$ = 149.6 ± 21.4 μM (Hill coefficient = 1.52 ± 0.15).
Microelectrophoretic Application of NMDA and AMPA to Spinal Neurons in Vivo

The ability of these glycineB antagonists to act as NMDA receptor antagonists in vivo was assessed by intravenous administration against responses of single neurons in the rat spinal cord to local microelectrophoretic application of AMPA and NMDA. The data shown are from 22 rats. The compounds were tested in a log 2, cumulative dose-progression (typically 0.5–16 mg/kg). Unfortunately, limited solubility in saline, characteristic for most compounds tested, made it difficult to test higher i.v. doses because large volumes (>1 ml) were required. More recent data indicate that solubility of choline derivatives for i.v. injection can be increased further by substituting 5.5% fructose for 0.9% NaCl.

The choline derivative of the mother compound MRZ 2/577 was tested over a range of i.v. doses (0.5–16 mg/kg), and only at the top doses (4–16 mg/kg) produced slight inhibition of responses of DH neurons to iontophoretic NMDA. This effect was dose-dependent (mean ID50, 33.7 ± 7.2 mg/kg; slope, 1.3 ± 0.3, n = 5) and statistically significant at 16 mg/kg (to 67 ± 5% control, P < .05, n = 5). No significant reduction of AMPA responses was observed at the doses tested (90 ± 3% control at 16 mg/kg, n = 5).

MRZ 2/502 was tested over a range of i.v. doses (0.5–8 mg/kg) and dose-dependently reduced responses of spinal DH neurons to iontophoretic NMDA (mean ID50, 1.6 ± 0.3 mg/kg, n = 6, fig. 6, table 3). Higher doses were required to reduce responses to iontophoretic AMPA (mean ID50, 5.5 ± 1.8 mg/kg, n = 6). The effects on both NMDA and AMPA developed within 20 to 30 s after injection, and the responses recovered quickly with a t1/2 of 8 to 15 min. Similar results were obtained with its water-soluble choline salt, MRZ 2/576 (figs. 6 and 7, table 3). This compound appeared to be somewhat less potent than MRZ 2/502 in reducing responses to NMDA (ID50, 2.8 ± 0.7 mg/kg) but was more selective (AMPA ID50 > 16 mg/kg). Thus, at top doses tested on each cell (2–8 mg/kg; mean, 4 mg/kg), NMDA responses were reduced to 30 ± 7% control, whereas responses to AMPA were reduced to 88 ± 6% control (n = 7). The true difference in potency of MRZ 2/502 and 2/576 is less than it appears because the choline derivative has a larger molecular weight. Thus, on a molar basis the potencies of MRZ 2/502 and 2/576 were not significantly different (6.1 ± 1.1 μmol/kg vs. 7.6 ± 1.9 μmol/kg, respectively; Mann-Whitney P > .05).

MRZ 2/514 was not tested because of solubility problems. However, its choline salt MRZ 2/570 was tested and produced a selective and dose-dependent inhibition of responses to NMDA of spinal DH neurons (fig. 7, table 3) with an ID50 of 4.5 ± 0.7 mg/kg. No reduction of the AMPA response was observed even at the top doses tested. (At 8–16 mg/kg; mean, 9 mg/kg, AMPA responses were 100 ± 9% control, cf. reduction of NMDA to 29 ± 4% control.)

Both MRZ 2/516 (0.5–8 mg/kg) and its water-soluble choline salt, MRZ 2/571 (0.5–16 mg/kg) dose-dependently antagonized responses of DH cells to exogenous NMDA (table 3). Again, the choline salt appeared to be less potent than the free acid (ID50 of MRZ 2/571 for the NMDA response was 4.7 ± 0.5 mg/kg, n = 6; for MRZ 2/516 it was 2.0 ± 0.3 mg/kg, n = 7). Both compounds substantially attenuated AMPA responses. Thus, at the top doses tested on each cell (mean, 4; range, 2–8 mg/kg), MRZ 2/516 reduced NMDA responses to 11 ± 2% control; responses of these cells to iontophoretic AMPA were 42 ± 10% control (n = 7). It was possible with these two compounds to calculate ID50 values for the AMPA response; they were 9.2 ± 1.1 mg/kg for MRZ 2/571 and 4.1 ± 1.4 mg/kg for MRZ 2/516.

Anticonvulsive Activity

The ability of Merz glycineB antagonists to act as NMDA receptor antagonists in vivo was confirmed by use of three convulsion models in rodents. All Merz glycineB antagonists inhibited MES- and PTZ-induced convulsions in mice with ED50 values ranging from 8 to 100 mg/kg i.p. (table 4). Most Merz compounds were also active against NMDA-induced convulsions although they appeared to be considerably less potent in this model. In the MES model, choline salts seemed to have a somewhat longer duration of action (fig. 8). Their anticonvulsive potency was increased after i.v. administration (table 5). MRZ 2/570 was somewhat less potent after s.c. administration, and was also active, although considerably less potent, after oral administration (table 5). At doses within the anticonvulsive range, myorelaxation (traction test) and ataxia (rotarod test) were observed. However, none of the Merz glycineB antagonists showed any serious acute toxicity, i.e., minimal lethal doses were all greater than 100 mg/kg (table 4). In contrast, of the standard compounds tested and despite their low nanomolar in vitro affinity for the glycineB site, only L-701,324 was systemically active and was not much more potent than the Merz compounds. Moreover, the TI of L-701,324 was actually worse than for the choline salts of the Merz compounds (see table 4).
The anticonvulsive effect of MRZ 2/570 (30 mg/kg) in the MES test was attenuated by very high doses of systemic glycine with an ED$_{50}$ = 688 mg/kg and by lower doses of D-cycloserine with an ED$_{50}$ = 8.8 mg/kg, full and partial agonist of the glycineB site, respectively, which indicates that their in vivo effects are also mediated at the glycine B site.

Probenecid (200 mg/kg) 30 min before administration of the tested agents prolonged considerably the duration of anticonvulsive action in the MES test. For example the half-lives of 2/514 and 2/570 30 mg/kg were about 40 and 80 min, respectively, in the absence of probenecid. In the presence of probenecid the half-lives were prolonged to about 180 and 210 min, respectively (fig. 8). Probenecid alone at the dose used (200 mg/kg) had no effect on MES-induced convulsions per se. Warfarin (50 mg/kg i.p.) was also able to slightly increase the potency of Merz glycineB antagonists in the MES test (table 6). However, it is not clear whether this reflects moderate binding to plasma albumins of the Merz glycineB antagonists because higher doses of warfarin (100 mg/kg) alone showed some anticonvulsive actions.

### Table 3

<table>
<thead>
<tr>
<th>Formulation</th>
<th>NMDA ED$_{50}$</th>
<th>Ti Tract</th>
<th>Ti Rot</th>
<th>PTZ ED$_{50}$</th>
<th>NMDA ED$_{50}$</th>
<th>Min Lethal</th>
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<tr>
<td>MRZ 2/499 Acid</td>
<td>87.6 (75.5–101.5)</td>
<td>1.2</td>
<td>1.5</td>
<td>18.6 (9.1–38.4)</td>
<td>57.5 (49.2–67.3)</td>
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<tr>
<td>MRZ 2/577 Choline salt</td>
<td>23.7 (13.3–42.2)</td>
<td>1.7</td>
<td>2.2</td>
<td>45.9 (39.5–53.4)</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>MRZ 2/502 Acid</td>
<td>47.6 (22.7–101.5)</td>
<td>0.5</td>
<td>0.6</td>
<td>8.3 (5.8–11.8)</td>
<td>26.2 (17.4–39.5)</td>
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<tr>
<td>MRZ 2/576 Choline salt</td>
<td>7.7 (6.0–9.8)</td>
<td>1.2</td>
<td>1.4</td>
<td>17.3 (15.0–19.9)</td>
<td>96.8 (89.4–104.7)</td>
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<td>MRZ 2/514 Acid</td>
<td>20.2 (8.4–48.7)</td>
<td>0.8</td>
<td>0.9</td>
<td>12.8 (9.2–17.9)</td>
<td>99.5 (95.1–104.0)</td>
<td>&gt;324</td>
</tr>
<tr>
<td>MRZ 2/570 Choline salt</td>
<td>12.8 (9.0–19.2)</td>
<td>1.0</td>
<td>1.4</td>
<td>10.6 (7.9–14.1)</td>
<td>58.3 (50.3–68.7)</td>
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<td>MRZ 2/516 Acid</td>
<td>16.6 (10.6–24.2)</td>
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<td>1.0</td>
<td>7.9 (4.3–14.7)</td>
<td>40.2 (32.1–50.3)</td>
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<tr>
<td>MRZ 2/571 Choline salt</td>
<td>15.5 (11.4–21.0)</td>
<td>1.1</td>
<td>1.1</td>
<td>12.7 (10.8–14.9)</td>
<td>59.3 (51.6–68.0)</td>
<td>&gt;324</td>
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<tr>
<td>L-701,324</td>
<td>4.1 (3.4–4.8)</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8 (0.6–1.2)</td>
<td>4.3 (2.7–6.8)</td>
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<td>L-701,252</td>
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<td>L-701,273</td>
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<td>L-689,560</td>
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<td>5,7-DCKA</td>
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<td>7-Ch-thio-KA</td>
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<tr>
<td>5,7-DiCl-thio-KA</td>
<td>&gt;20.0</td>
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### Table 4

<table>
<thead>
<tr>
<th>Formulation</th>
<th>NMDA ID$_{50}$</th>
<th>AMPA ID$_{50}$</th>
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<tbody>
<tr>
<td>MRZ 2/499 Acid</td>
<td>33.7 ± 7.2</td>
<td>&gt;32</td>
</tr>
<tr>
<td>MRZ 2/577 Choline salt</td>
<td>1.6 ± 0.3</td>
<td>5.5 ± 1.8</td>
</tr>
<tr>
<td>MRZ 2/502 Acid</td>
<td>2.8 ± 0.7</td>
<td>&gt;16</td>
</tr>
<tr>
<td>MRZ 2/576 Choline salt</td>
<td>4.5 ± 0.7</td>
<td>4.1 ± 1.4</td>
</tr>
<tr>
<td>MRZ 2/514 Acid</td>
<td>2.0 ± 0.3</td>
<td>7</td>
</tr>
<tr>
<td>MRZ 2/570 Choline salt</td>
<td>4.7 ± 0.5</td>
<td>9.2 ± 1.1</td>
</tr>
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</table>

**Fig. 7.** Pooled data on the effects of i.v. MRZ 2/576 (0.5–4 mg/kg, n = 6), MRZ 2/570 (1–8 mg/kg, n = 5) and MRZ 2/571 (1–8 mg/kg, n = 6) on responses to iontophoretic NMDA and AMPA in the spinal DH of α-chloralose-anesthetized rats. Examples of experimental protocol are shown in figure 6. Data are presented as mean percentages of control values ± S.E. Significant difference from control is shown as *P < .05; **P < .01, Mann-Whitney test.
Reflex (Tract.) impairment or rotarod failure (Rot.) divided by the ED50 for MES-phthalazine-dione derivatives studied are moderately potent.

Increased their potency against MES-induced seizures.

Albumins

Warfarin was also able to increase the potency of Merz glycine B.

**Table 6**

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>ED50 mg/kg</th>
<th>Tracta TI</th>
<th>Rot.a TI</th>
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</thead>
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<tr>
<td>MRZ 2/570 i.v.</td>
<td>9.5 (5.5–16.4)</td>
<td>0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>MRZ 2/570 i.p.</td>
<td>12.8 (9.0–18.2)</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>MRZ 2/570 s.c.</td>
<td>18.6 (11.5–30.0)</td>
<td>0.7</td>
<td>1.0</td>
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<tr>
<td>MRZ 2/570 p.o.</td>
<td>69.9 (34.0–143)</td>
<td>NT</td>
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</table>

The therapeutic index (TI) was calculated as the ED50 for inhibition of traction reflex (Tract.) impairment or rotarod failure (Rot.) divided by the ED50 for MES-induced seizures per se.

**Table 5**

MRZ 2/570 was more potent against MES-induced seizures after i.v. administration but was less potent after s.c. or p.o. administration.

**Discussion**

The present data illustrate that the tricyclic pyridophthalazine-dione derivatives studied are moderately potent and selective glycineB antagonists in vitro. Most compounds displaced [3H]DCKA, [3H]MDL-105,519 and [3H]glycine binding to rat cortical membranes with high nanomolar affinity and antagonized steady-state inward current responses of cultured hippocampal neurons to NMDA with high nanomolar to low micromolar affinity. The NMDA receptor antagonism observed was typical for glycineB antagonists, i.e., showed competition with glycine, revealed glycine-dependent desensitization (Mayer et al., 1989a,b; Lerma et al., 1990, Vyklicky et al., 1990; Parsons et al., 1993) and was not use- or voltage-dependent. Much higher concentrations were required to antagonize steady-state inward current responses to AMPA and, in this case, the antagonism observed was reminiscent of a competitive interaction (Parsons et al., 1994). These effects on AMPA receptors agree closely with provisional unpublished data which indicate a least a 100-fold lower affinity in displacing [3H]AMPA binding (the Ki values of MRZ 2/502, 2/514 and 2/516 were 24 ± 7 μM, 33 ± 9 μM and 26 ± 5 μM, respectively), but no activity up to 10 μM on many other, well characterized CNS receptors (other ionotropic glutamate receptor recognition sites and several subtypes of adenosine, adrenergic, dopamine, GABA, serotonin, opoid, histamine, cholinergic, purinergic and sigma receptors and voltage-activated channels; Pan Labs, Bothell, WA). These Merz glycineB antagonists were also tested against glutamate (100 μM)-induced neurotoxicity in cultured cortical neurons and provided near complete protection at 30 μM (data not shown).

The ability of these glycineB antagonists to act as NMDA receptor antagonists in vivo was assessed by i.v. administration against responses of single neurons in the rat spinal cord to microelectrophoretic application of AMPA and NMDA. They were potent NMDA receptor antagonists in vivo with ID50 values in the low milligram per kilogram range. On a molar basis these in vivo doses are about 20-fold higher than those required for NMDA receptor antagonism in vitro, which indicates relatively good penetration to the CNS. Somewhat higher doses of the free acids also antagonized responses to AMPA. However, two of the choline derivatives were more selective; this apparent lack of selectivity contrasts with the in vitro assays. As such, it seems that at least some of the observed effects on AMPA may be secondary to depression of NMDA receptor-mediated background activity (Chizh et al., 1996).

Merz glycineB antagonists also inhibited PTZ-, NMDA- and MES-induced convulsions in mice after i.p. administration. They were most potent against MES- and PTZ-induced convulsions but less potent against NMDA-induced convulsions. The reason for these differences remains unclear. We have extensive data that the MES test is a good index for NMDA receptor antagonism in vivo (Parsons et al., 1995), and this test was therefore used for further characterization of the in vivo activity of Merz glycineB antagonists.

As exemplified by MRZ 2/570, Merz glycineB antagonists were found to be most potent against MES-induced convulsions after i.v. administration, somewhat less active after i.p. and s.c. administration and least potent after p.o. administration. Their anticonvulsant actions were reversed by both systemic glycine and D-cycloserine administration, which indicates that their in vivo effects are also mediated at the glycineB site. The duration of anticonvulsive action after i.p. administration was rather short (30–40 min) but was pro-
longed by probenecid, which indicates rapid transport out of the brain by the organic acid transporter in the choroid plexus (Moroni et al., 1988; Leeson and Iverson, 1994; Santomaria et al., 1996). Warfarin was able to slightly increase their potency, which suggests only moderate binding to plasma albumins. Moreover, higher doses of warfarin alone also had anticonvulsive activity, which indicates that this potentiation may actually have been caused by synergistic interactions. These compounds had a similar in vivo potency to the standard glycine_B antagonist L-701,324 despite being at least 20-fold less potent as NMDA receptor antagonists in vitro. Taken together with the relatively modest effects of warfarin, this would tend to indicate that plasma albumin binding of these Merz glycine_B antagonists is much less than seen previously with other glycine_B antagonists (T. Priestley, personal communication). This fact might partially account for their much improved pharmacokinetics, i.e., penetration to the CNS.

Initial results from microdialysis studies indicate that MRZ 2/570 reaches peak extracellular concentrations in the brain of about 1.7 μM at 20 min after i.p. administration of 30 mg/kg in rats. The half-life of MRZ 2/570 in the rat brain reflects that seen for anticonvulsive activity in mice and can also be prolonged by probenecid (200 mg/kg i.p.) (peak concentrations of 2.6 μM with a half-life of about 90 min; M. Hesselink, unpublished data).

The fact that most of the very high-affinity, standard glycine_B receptor antagonists tested in the present study were inactive in vivo indicates that attempts to improve systemic activity solely by increasing the in vitro potency of glycine_B antagonists with poor pharmacodynamic properties may be the wrong approach. Moreover, the ability of some full antagonists of the glycine_B site to unmask NMDA receptor glycine-sensitive desensitization may underlie their promising therapeutic profile (Parsons et al., 1993) and seems, in part, to be inversely related to affinity. The present data show a trend toward a greater antagonism of steady-state than peak inward current responses to NMDA by lower affinity glycine_B full antagonists, i.e., the lower affinity compounds induced a greater degree of glycine-sensitive desensitization (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Mayer et al., 1989a,b; Lerma et al., 1990, Vyklinczy et al., 1990; Parsons et al., 1993). This finding is in line with a recent report by Molnar and Erdö (1996). In contrast, the partial agonists (+R)-HA-966 and β-cycloserine showed no differentiation between peak and steady-state components despite relatively low affinity. ACPC behaved essentially like a full agonist, with higher concentrations causing antagonism, probably via effects at a different recognition site. Similar differences in the profiles of some partial agonists and full antagonists have also been reported previously and attributed to differential effects on allosteric interactions between the agonist and glycine_B sites (Kemp and Priestley, 1991; Grimwood et al., 1995).

Receptor desensitization may represent a physiological process serving as an endogenous control mechanism to prevent long-term neurotoxic activation of glutamate receptors but allow their transient physiological activation (Parsons et al., 1993). Ischemia increases not only the concentration of extracellular glutamate but also that of glycine, and although this later effect is less pronounced, it actually persists for much longer (Globus et al., 1991). Hence, some full glycine_B antagonists could restore normal synaptic transmission under excitotoxic conditions by increasing NMDA receptor desensitization to its physiological level (Parsons et al., 1993). Indeed, our own provisional data indicate these Merz glycine_B antagonists are neuroprotective against NMDA-induced lesion of the nucleus basalis fo Meynert in rats as well as in a global ischemia model in gerbils (Danyssz et al., 1996).

Doses within the anticonvulsive range also produced myorelaxation and ataxia. It should be stressed, however, that the MES test was used purely as an index of NMDA receptor antagonism in vivo and not as a preclinical model for the possible utility of Merz glycine_B antagonists against generalized seizures. The relatively poor therapeutic indices in the MES test do not necessarily contradict the hypothesis discussed above. Although the biophysical properties of some glycine_B full antagonists may allow therapeutically relevant concentrations to block chronic, low-level pathological activation of NMDA receptors while leaving their synaptic activation intact, precisely these properties may also underlie the poor therapeutic indices relative to antiepileptic activity seen in the present study because of the synaptic nature of both seizures and normal glutamatergic transmission. This in turn does not mean that poor therapeutic indices can be expected in all models of disturbed glutamatergic neurotransmission. The literature indicates that some glycine_B antagonists have much improved therapeutic indices than others belonging to a similar class. For example, MDL 100,458 and MDL 102,288 are equipotent as glycine_B antagonists in vitro but exhibit strikingly different in vivo profiles in audiogenic seizures in DBA/2 mice and in separation-induced ultrasonic vocalizations in rat pups, a model of anxiety activity (Kehne et al., 1995). The reason for these differences is not clear, although the compounds may possibly act preferentially at different NMDA receptor subtypes (Danyssz et al., 1995).

Our own data also indicate that these Merz glycine_B antagonists may have improved potency and therapeutic indices in some indications and show a very different behavioral profile to competitive and noncompetitive NMDA receptor antagonists. For example, in rats in the open-field test they actually attenuate the hyperlocomotion induced by both PCP and amphetamine at nonataxic doses and show no disruption of prepulse inhibition (Danyssz et al., 1996; see also Bristow et al., 1995, 1996). These data indicate a lack of psychotomimetic potential and possibly even antipsychotic activity of these tricyclic pyrido-pthalazine-diones. Furthermore, they antagonize morphine place preference in rats at low, nonataxic doses, which suggests therapeutic potential in the treatment of opioid abuse (Danyssz et al., 1996). MRZ 2/570 (5–10 mg/kg i.p.) has no negative effects on working or reference memory in the radial maze (W. Danyssz, unpublished data). Finally, even very high doses of up to 100 mg/kg i.p. MRZ 2/576 does not cause any neurodegenerative changes in the cingulate or retrosplenial cortex of female or male rats (A. Schwiauer, unpublished data).

We have now initiated extensive testing of these glycine_B antagonists in preclinical models of various disorders that have been associated with disturbances of the glutamatergic system. These compounds could be useful in the treatment of the following disorders: 1) acute excitotoxicity such as ischemia during stroke, trauma, hypoxia, hypoglycemia and hepatic encephalopathy; 2) chronic neurodegenerative dis-
eases such as Alzheimer's disease, vascular dementia, Parkinson's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis, AIDS-neurodegeneration, olivopontocerebellar atrophy, Tourette's syndrome, motor neuron disease, mitochondrial dysfunction, Korsakoff syndrome, Creutzfeldt-Jakob disease; 3) other disorders related to long-term plastic changes in the CNS such as chronic pain, drug tolerance, dependence and addiction (e.g., opioids, cocaine, benzodiazepines and alcohol); 4) epilepsy (partial complex seizures), tardive dyskinesia, schizophrenia, anxiety, depression, visceral pain, spasticity and tinnitus.

In conclusion, these tricyclic pyrido-phthalazine-diones represent a novel class of systemically active glycineNMDA antagonists with much improved penetration to the CNS. They should prove to be useful tools to elucidate the therapeutic potential of this class of NMDA receptor antagonists in various disorders that involve disturbances of glutamatergic transmission.

References


LOCHNER, W., WILZ, P., RUNDFELDT, C., BARAN, H. AND HONACK, D.: Anticonvul- sant effects of the glycine/NMDA receptor ligands D-cycloserine and D-


MURATA, S. AND KAWASAKI, K.: Common and uncommon behavioural effects of systemic active glycineB antagonists 1275


MURATA, S. AND KAWASAKI, K.: Common and uncommon behavioural effects of systemic active glycineB antagonists 1275


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