ELSEVIER

European Journal of Pharmacology 342 (1998) 39–46

ejp

Weak anticonvulsant effects of two novel glycine$_B$ receptor antagonists in the amygdala-kindling model in rats

Piotr Wlaź *, Wolfgang Löscher

Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany

Received 23 June 1997; revised 17 October 1997; accepted 21 October 1997

Abstract

In the present work we evaluated the anticonvulsant effects of two novel antagonists of the glycine co-agonist site (glycine$_B$ receptor) within the *N*-methyl-D-aspartate (NMDA) receptor complex, MRZ 2/576 (a tricyclic pyrido-phtalazin dione derivative) and L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)quinoline). As a model of epilepsy we used amygdala-kindled rats, which are considered as a model to study the efficacy of drugs against human complex partial seizures. MRZ 2/576 (2.5–10 mg/kg i.p. 15 min before testing) did not influence afterdischarge threshold, which is believed to be the most subtle indicator of efficacy against kindled seizures, nor did it affect other measures of seizure activity such as seizure severity, seizure duration and afterdischarge duration. However, MRZ 2/576 produced dose-dependent ataxia as measured in the open field and rotarod test. The highest dose tested (10 mg/kg) also markedly reduced rectal temperature (by about 1.5°C). L-701,324 (2.5–10 mg/kg i.p. 30 min before testing) dose dependently and significantly increased afterdischarge threshold, but other seizure parameters remained unchanged. The ataxia produced by lower doses of L-701,324 (2.5 and 5 mg/kg) was more pronounced than that caused by MRZ 2/576. However, the ataxia observed following the higher dose of L-701,324 (10 mg/kg) was less intense than that elicited by MRZ 2/576. The behavioral alterations produced by the two drugs did not resemble those characteristic for classical competitive and uncompetitive NMDA receptor antagonists. In conclusion, our data indicate that glycine$_B$ receptor antagonists are not promising candidates for the treatment of complex partial seizures in humans, at least as monotherapy. © 1998 Elsevier Science B.V.

Keywords: Glutamate; NMDA; MRZ 2/576; L-701,324; Kindling; (Rat)

1. Introduction

Glutamate, the main excitatory amino acid, operates by interacting with two major families of its receptors: metabotropic and ionotropic receptors. The latter class can be further divided into three subtypes, named after their prototype selective agonists, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), and kainate receptors (Scatton, 1993). The NMDA receptor is the best characterized receptor subtype for excitatory amino acids. It is the ligand-gated ion channel that mediates sustained excitatory synaptic transmission (Scatton, 1993). Antagonists at this receptor, both competitive and uncompetitive (channel blockers), exhibit distinct anticonvulsant activity. They are effective in a variety of animal models of epilepsy (Croucher et al., 1982; Czuczwar and Meldrum, 1982; Meldrum et al., 1983; Löscher et al., 1988; Schmutz et al., 1990). Unhappily, both categories of NMDA receptor antagonists induce severe unwanted effects at doses close to or even lower than those found to be anticonvulsant, which seriously limits their usefulness (Löscher, 1993). Furthermore, they are of limited value against kindled seizures in rats because of their low efficacy and propensity to induce pronounced psychotomimetic-like behaviors in these rats (Löscher and Hönack, 1991a). The discovery by Johnson and Ascher in 1987 that the activity of glutamate at the NMDA receptor is modulated by glycine (Johnson and Ascher, 1987) has opened an intriguing possibility that the NMDA receptor can be antagonized indirectly at the strychnine-insensitive glycine site. Compounds with an antagonist action at the glycine site have since been synthesized and their anticonvulsant activity has been reported (Croucher and Bradford, 1990; Baron et al., 1990; Croucher and Bradford, 1991). However, only quite recently have

* Corresponding author. Department of Pharmacology, Faculty of Veterinary Medicine, Agricultural University, P.O. Box 158, PL-20-950 Lublin, Poland. Tel.: +48-81-4456568; fax: +48-81-4456565; e-mail: wlaz@ursus.ar.lublin.pl

glycine$_B$ receptor antagonists with anticonvulsant activity after systemic administration been developed (Rowley et al., 1993; Kulagowski et al., 1994; Kehne et al., 1995; Ilyin et al., 1996).

About 70% of cases of complex partial seizures in adults are resistant to currently available therapies (Juul-Jensen, 1986). As amygdala-kindling model in rats has been postulated as a model to study complex partial seizures with secondary generalization (Löscher and Schmidt, 1988), the aim of this study was to assess the anticonvulsant and adverse effect potential of the novel glycine$_B$ receptor antagonist MRZ 2/576 (a tricyclic pyridophtalazin dione derivative; Danysz et al., 1996) in this model. For comparison we used L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)quinoline), for which anticonvulsant and behavioral profiles and selectivity as an antagonist for the glycine$_B$ receptor have been described (Bristow et al., 1996a,b).

2. Materials and methods

2.1. Animals

Female Wistar rats (Harlan–Winkelmann, Borchen), weighing 250–300 g, were used in this study after at least one week of acclimatization. The animals were housed singly in Makrolon cages under controlled environmental conditions (ambient temperature 24–25°C; relative humidity 50–60%; 12/12 h light/dark cycle, light on at 6:00 a.m.). Standard laboratory chow pellets (Altromin 1324 standard diet) and tap water were freely available. All experiments were done at the same time of day (between 9.00 a.m. and 12.00 p.m.) to minimize circadian influences on seizure susceptibility.

2.2. Electrode implantation and kindling

The rats were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) and received stereotaxic implantation of one bipolar electrode in the right basolateral amygdala. The electrode consisted of two twisted Teflon-coated stainless steel wires (250 μm in diameter), separated at the tip by 0.5 mm. The following stereotaxic coordinates were used: AP −2.2 mm, L −4.8 mm, V −8.5 mm according to the brain atlas of Paxinos and Watson (1986). All coordinates were measured from bregma. One screw, placed over the left parietal cortex, served as the indifferent reference electrode; two others were screwed in the skull to anchor the electrode assembly. Both bipolar and reference electrodes were connected to miniature female plugs, and the assembly was fixed on the skull with dental acrylic cement. After electrode implantation the animals were treated with antibiotics for one week to prevent infection.

After a post-operative period of two weeks, constant current stimulations (500 μA, 1 ms, monophasic square-wave pulses, 50 Hz for 1 s) were delivered to the amygdala once daily (five times per week) until at least 10 sequential fully kindled stage-5 seizures were elicited. Seizure severity was scored according to a modified Racine's scoring system (Racine, 1972): 1, immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus; 2, head nodding associated with more severe facial clonus; 3, clonus of one forelimb; 3.5, bilateral forelimb clonus without rearing; 4, bilateral forelimb clonus accompanied by rearing; 4.5, falling on a side (without rearing), loss of righting reflex accompanied by generalized clonic seizures; 5, rearing and falling on the back accompanied by generalized clonic seizures. Seizure duration was the duration of limbic seizures (stage 1–2) and/or motor seizures (stage 3–5); the limbic seizure activity, which sometimes occurred after termination of motor seizures was not included in seizure duration. Afterdischarges were defined as spikes with a frequency of at least 1 Hz and amplitude at least twice the pre-stimulation baseline present in the electroencephalogram (EEG) recorded from the site of stimulation. High-amplitude afterdischarges (usually accompanied by generalized seizure activity) were frequently followed by low-amplitude afterdischarges. Because of the lack of general consensus as to the nature of these afterdischarges, afterdischarge duration described in the present study refers to high-amplitude spiking. Secondary afterdischarges, which in most cases follow primary afterdischarges generated by the epileptic focus (Ebert et al., 1995), were not included in afterdischarge duration.

2.3. Evaluation of anticonvulsant effects

Afterdischarge threshold was evaluated in 6–7 fully-kindled rats. Afterdischarge threshold was determined after administration of a test drug or its vehicle by administering a series of stimulations at intervals of 1 min, increasing in steps of about 20% of the previously applied current until an afterdischarge lasting at least 3 s was evoked. Electrical stimulations for each individual animal began about three 20%-steps below the individual control afterdischarge threshold value measured 2–3 d before the drug experiment. At the afterdischarge threshold current, seizure severity, seizure duration and afterdischarge duration were recorded and analyzed.

2.4. Evaluation of adverse effects

Rectal temperature was measured thrice: shortly before administration of the compounds or their vehicles (after about 15 min adaptation to the laboratory environment), in the middle of the pretreatment time, and just before stimulation. Behavioral alterations were scored in the open field and in the rotarod test (see below) twice, i.e. in the middle of the pretreatment time and shortly before stimulation. Ataxia was evaluated according to the 6-point scoring

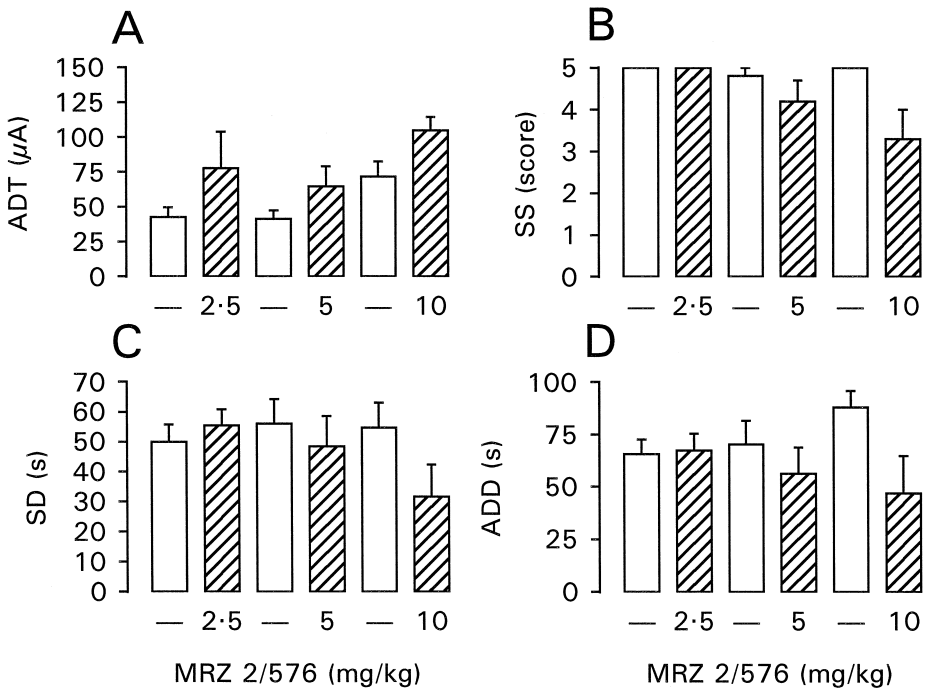

Fig. 1. Effect of MRZ 2/576 on afterdischarge threshold (ADT, panel A), and seizure severity (SS, panel B), seizure duration (SD, panel C), and afterdischarge duration (ADD, panel D) recorded at afterdischarge threshold in six fully kindled rats. Data are means + S.E.M. MRZ 2/576 (hatched bars) or its vehicle (open bars) was administered i.p. 15 min before the test. Control readings, from the same group of rats, were obtained 2–3 d before the respective drug experiments. Afterdischarge threshold was determined by applying a series of stimulations, spaced by 1 min, that increased in steps of about 20% of the previous current until an afterdischarge lasting at least 3 s was evoked. Absence of the error bars indicates lack of variance.

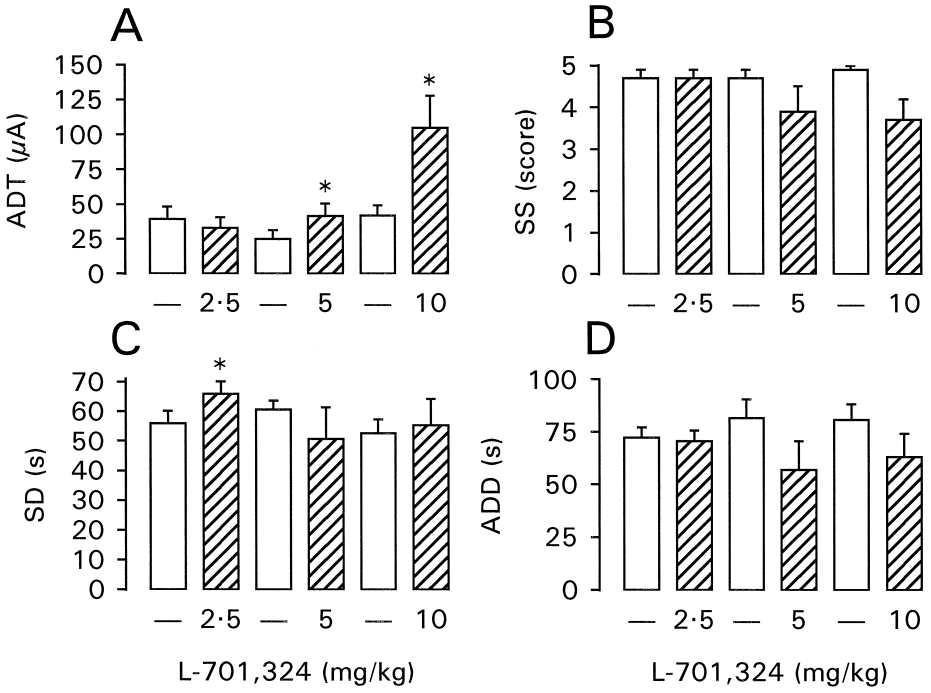

Fig. 2. Effect of L-701,324 on afterdischarge threshold (ADT, panel A), and seizure severity (SS, panel B), seizure duration (SD, panel C), and afterdischarge duration (ADD, panel D) recorded at afterdischarge threshold in seven fully kindled rats. Data are means + S.E.M. L-701,324 (hatched bars) or its vehicle (open bars) was administered i.p. 30 min before the test. For further details refer to the legend to Fig. 1. Significant difference versus respective control (Wilcoxon signed-rank test for paired replicates) is indicated by asterisk ($P < 0.05$).

system (cf., Löscher and Hönack, 1991b): (1) slight ataxia (tottering of hind limbs), (2) more pronounced ataxia (dragging of hind limbs), (3) further increase of ataxia (more pronounced dragging of hind limbs), (4) marked ataxia (animals only occasionally lose balance during forward locomotion), (5) very marked ataxia (animals frequently lose balance during forward locomotion), and (6) animals, despite attempts, are not able to move forward. In addition, the rats were subjected to the rotarod test. In this test, the rats had to maintain their equilibrium on a rotating, polypropylene foam-coated rod (5 cm in diameter, 8 rpm) for at least 1 min. If a rat fell from the rod, it was placed on the rod again. Only animals that fell from the rod in each of three consecutive 1 min attempts were considered to exhibit motor impairment.

2.5. Drugs

MRZ 2/576 (Merz, Frankfurt/Main) was freshly dissolved in distilled water (final pH 8–9); L-701,324 (kindly supplied by Merck, Sharp and Dohme, Harlow) was first dissolved in polyethylene glycol 400 (PEG 400, Sigma Chemical, St. Louis, MO) and then diluted with distilled water to the final PEG 400 concentration of 10–20%, depending on the concentration of the drug, and slightly alkalinized with NaOH (final pH ~8). Rats were dosed intraperitoneally (i.p.) with MRZ 2/576 and L-701,324 15 and 30 min before the test, respectively. The volume of all administered solutions was 2–3 ml/kg b.w. Control experiments were performed following administration of the respective vehicles.

2.6. Data analysis

All data are presented as means ± S.E.M. Statistical significance of differences between seizure readings in the same group of animals was calculated by the Wilcoxon signed-rank test for paired replicates. Data from the open field (ataxia) and rotarod test were compared by using Mann–Whitney *U* test and Fisher's exact probability test, respectively. Differences in rectal temperature were analyzed by means of repeated measures two-way analysis of variance (ANOVA) and the Student–Neuman–Keuls post hoc test. A P value less than or equal to 0.05 was considered to be statistically significant. All tests were two-tailed. The calculations were performed with SigmaStat for Windows 1.0 (SPSS ASC, Erkrath).

3. Results

3.1. Effect of MRZ 2/576 and L-701,324 on focal seizure threshold in amygdala-kindled rats

MRZ 2/576 (range 2.5–10 mg/kg i.p., 15 min before the test) did not significantly influence the focal seizure threshold in kindled rats (Fig. 1A). Other parameters of kindled seizures, namely seizure severity, seizure duration and afterdischarge duration, were also not changed (Fig. 1B, C, D). It should be, however, noted that there was a tendency towards threshold-increasing activity (Fig. 1A), but a clear-cut disruption of motor function precluded further increases of the dose (see Section 3.2).

As shown in Fig. 2A, L-701,324 (range 2.5–10 mg/kg i.p., 30 min before the test) produced a dose-dependent increase in afterdischarge threshold. Seizure severity, seizure duration and afterdischarge duration remained unaffected by treatment with L-701,324 (Fig. 2B, C, D) except that seizure duration following administration of L-701,324 at the lowest dose (2.5 mg/kg) was slightly longer.

The pre-stimulation EEGs recorded after administration of both MRZ 2/576 and L-701,324 were not different when compared to those recorded during control sessions.

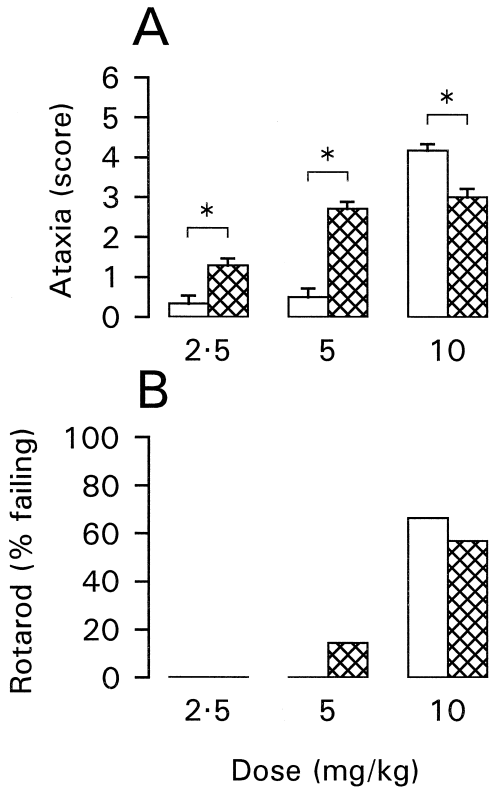

Fig. 3. Adverse effects produced by MRZ 2/576 (open bars) and L-701,324 (double-hatched bars) in amygdala-kindled rats. Motor impairment was quantified in the open field (panel A) and in the rotarod test (panel B). Data from the open field test are presented as means + S.E.M for six (MRZ 2/576) or seven (L-701,324) animals and were measured immediately before afterdischarge threshold determinations. Data from the rotarod test are presented as the percentage of animals from the respective groups that failed this test. Significant differences between respective MRZ 2/576- and L-701,324-treated groups (Mann–Whitney *U* test) are indicated by asterisks ($P < 0.05$).

3.2. Behavioral effects of MRZ 2/576 and L-701,324 in kindled rats

MRZ 2/576 over the dose-range tested produced hypolocomotion but did not induce stereotyped behavior in kindled rats. The first signs of motor impairment were already seen after 2–3 min following injection of MRZ 2/576. The ataxiogenic properties of MRZ 2/576 are depicted in Fig. 3A; the tested substance already at the lowest dose (2.5 mg/kg) produced slight motor impairment, as quantified in the open field, and this impairment was much more pronounced when the dose was further increased to 10 mg/kg, culminating in a complete loss of the righting reflex when the animals were injected with 20 mg/kg (data not illustrated). The rotarod test indicated motor impairment in 66% of the animals at a dose of 10 mg/kg (Fig. 3B). Rectal temperature was markedly and time dependently reduced after injection of MRZ 2/576 at a dose of 10 mg/kg ($F = 132.4$, treatment, $P < 0.0001$; Fig. 4).

Similarly to MRZ 2/576, L-701,324 over the dose-range tested produced hypolocomotion but did not induce stereotyped behavior in kindled rats. L-701,324 at doses that were able to increase afterdischarge threshold dose dependently produced ataxia (starting at 2.5 mg/kg) and impaired performance in the rotarod test (Fig. 3A, B). The ataxia produced by L-701,324 at lower doses (2.5–5 mg/kg) was more pronounced than that produced by MRZ 2/576 at these doses ($P < 0.05$). However, MRZ 2/576, 10 mg/kg, caused more pronounced ataxia than L-701,324 administered at the same dose ($P < 0.05$). The maximal intensity of adverse effects was observed 30 min post-injection. Changes in rectal temperature following administration of L-701,324 were inconsistent (Fig. 4). After a dose of 2.5 mg/kg there was a slight but significant increase in rectal temperature after 30 min ($F = 32.81$, treatment, $P = 0.0012$). Higher doses of L-701,324 (5 and 10 mg/kg) did not affect body temperature.

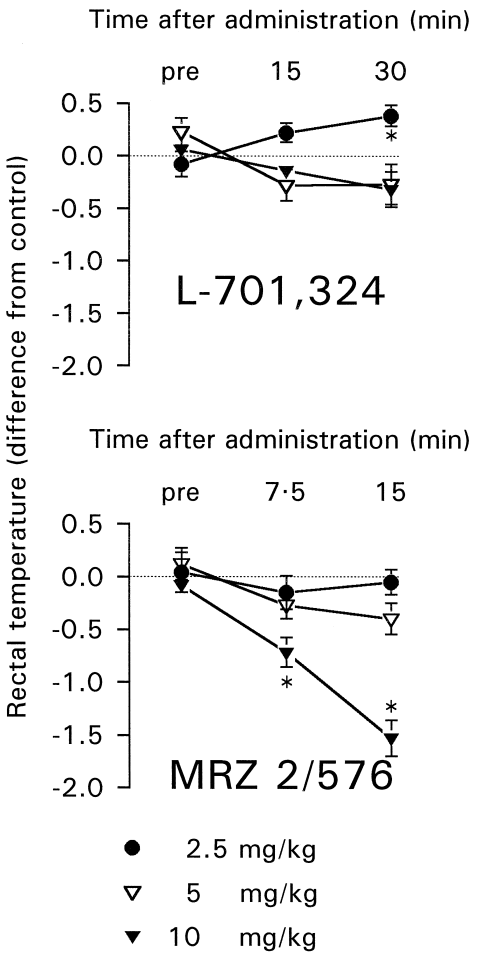

Fig. 4. Changes in rectal temperature following administration of MRZ 2/576 (lower panel) or L-701,324 (upper panel) in amygdala-kindled rats. Data are shown as differences (means ± S.E.M.) between rectal temperatures recorded after vehicle administration at respective time points 2–3 d before drug sessions and those recorded during drug sessions ($n = 6–7$). Data were analyzed by two-way repeated measures analysis of variance. Significant difference versus respective time point during control experiment (Student–Neuman–Keuls post-hoc test) is indicated by asterisk ($P < 0.05$).

4. Discussion

Glutamatergic neurotransmission undoubtedly plays a pivotal role in the initiation and propagation of seizure activity in the brain (Rogawski, 1995). As a logical consequence of this assumption, it has been postulated that agents which compromise glutamate-mediated excitatory input may be effective antiepileptic drugs. Indeed, they display considerable efficacy in many animal models of epilepsy (Löscher et al., 1988; Patel et al., 1990; Schmutz et al., 1990; Smith et al., 1993; Wlaź et al., 1994a). In contrast, in the rat kindling model of human partial seizures (limbic epilepsy), both competitive and uncompetitive (channel blockers) NMDA receptor antagonists exhibit weak, if any, anticonvulsant action (Löscher and Schmidt, 1988; McNamara et al., 1988; Löscher and Hönack, 1991b; Cotterell et al., 1992). Furthermore, they induce severe psychotomimetic-like adverse effects in kindled rats usually at doses that are devoid of significant anticonvulsant effects (Löscher and Hönack, 1991b). This was confirmed during a clinical trial in which epileptic patients had to be prematurely withdrawn from therapy with the competitive NMDA receptor antagonist, D-CPPene (D(−)(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid), because of severe psychotic and motor impairing side effects (Sveinbjornsdottir et al., 1993). Thus, this negative clinical experience in conjunction with preclinical data reported earlier by Löscher and Hönack (1991a) substantiate that investigations conducted with kindled rats may be of critical value as far as the efficacy and adverse effect potential of drugs in human patients with complex partial seizures

are concerned. The fact that (1) glycine$_B$ receptor stimulation is now recognized as an absolute requirement for activation of the NMDA receptor-coupled channel (Kleckner and Dingledine, 1988), and (2) glycine levels usually dramatically increase during epileptic seizures in both animal models (Globus et al., 1991; Löscher et al., 1993) and in epileptic patients (Carlson et al., 1992; Ronne-Engström et al., 1992) support the belief that glycine$_B$ receptor antagonists could be superior to NMDA receptor antagonists as antiepileptics. Indeed, the glycine$_B$ receptor antagonist, 7-chlorokynurenic acid, when administered intracerebroventricularly retards amygdala-kindling development (Namba et al., 1993) and suppresses amygdala-kindled seizures in rats (Rundfeldt et al., 1994) at doses not associated with apparent behavioral deficits.

In the present study our attention was focused on the anticonvulsant effects of two novel antagonists at the strychnine-insensitive co-agonist site on the NMDA receptor/ionophore complex. MRZ 2/576 and L-701,324 have a good bioavailability and were recently reported to exhibit distinct anticonvulsant effects (Bristow et al., 1996b; Parsons et al., 1997). In mice, these compounds were active against audiogenic seizures and convulsions induced by electroshock, pentylenetetrazol and NMDA/*N*-methyl-D,L-aspartate (NMDLA) (Bristow et al., 1996b; Parsons et al., 1997). In rats, L-701,324 dose dependently suppressed pentylenetetrazol-induced tonic seizures (Bristow et al., 1996b). It is worth noting that the anticonvulsant activity of L-701,324 depended on the route of administration in that, for instance, this agent was about 20-fold less potent in the pentylenetetrazol test when it was administered orally instead of by the intravenous route (Bristow et al., 1996b). Accordingly, we decided to apply both compounds by the i.p. route to enable direct comparison.

L-701,324 dose dependently increased afterdischarge threshold but this effect was paralleled by marked side effects. This is inconsistent with our previous report, where doses of 2–10 mg/kg were unable to increase the afterdischarge threshold (Ebert et al., 1997). One possible explanation for this discrepancy can be the age of our animals. The rats used in the present study were substantially younger than those previously used and it has been reported that [^{3}H]-glycine binding sites are severely decreased in number in the telencephalic regions, including the hippocampus and cerebral cortex, in aged rats, as studied by in vitro autoradiography (Kito et al., 1990). The remaining seizure measures were not changed, which is in line with our previously published data (Ebert et al., 1997). MRZ 2/576 was devoid of any anticonvulsant activity. It should be stressed that both substances used in this study caused marked and dose-dependent motor deficits, indicating that pharmacologically relevant doses were tested. Moreover, as shown by microdialysis in rats, MRZ 2/576 20 min after i.p. administration of 10 mg/kg reaches concentrations of about 0.8 μM, thus approaching concentrations within the IC$_{50}$ values for the glycine$_B$ receptor (Danysz, personal communication). MRZ 2/576 and L-701,324 did not produce the phencyclidine (PCP)-like behavioral syndrome of hyperlocomotion, stereotypies and ataxia which is characteristic for the 'classical' uncompetitive and competitive NMDA receptor antagonists (cf., Löscher and Hönack, 1991b). As mentioned above, kindled rats are especially prone to exhibit these behavioral effects (Löscher and Hönack, 1991a). Thus, this is in agreement with other reports demonstrating that glycine$_B$ receptor antagonists and low-efficacy partial antagonists do not preferentially induce PCP-like behavioral sequelae (Löscher et al., 1994; Tricklebank et al., 1994; Bristow et al., 1996b; Ebert et al., 1997) or generalize to the PCP discriminative stimulus (Witkin et al., 1997).

It is known that both the uncompetitive antagonist dizocilpine (MK-801) and the glycine$_B$ receptor low-efficacy partial agonist (+)HA-966 (*R*-(+)-3-amino-1-hydroxypyrrolid-2-one) produce alterations in the EEG that are reminiscent of paroxysmal epileptic activity (Wlaź et al., 1994b; Tortella and Hill, 1996). Special emphasis should be placed on the fact that these functional changes were observed in kindled rats (Wlaź et al., 1994b) but not in non-kindled ones (Tortella and Hill, 1996), which again indicates that epileptogenesis, as revealed by kindling, increases the sensitivity to the adverse effects of compounds that affect glutamatergic transmission. However, none of the presently tested drugs changed the resting (prestimulus) electroencephalographic pattern.

In conclusion, these experiments demonstrated that MRZ 2/576 is devoid of significant anticonvulsant activity in the kindling model of epilepsy in rats. The equivocal efficacy of L-701,324 in the kindling model (present study; Ebert et al., 1997) indicates the need for further studies to answer the question whether or not compounds acting at the glycine$_B$ receptor are promising candidates for antiepileptic drugs. Recent data show that a greater efficacy without concomitant potentiation of adverse effects in kindled rats can be achieved by joint administration of glycine$_B$ receptor ligands with substances affecting other modulatory domains of the NMDA complex, like polyamine site antagonists (Ebert et al., 1997; Wlaź et al., in preparation), or with conventional antiepileptic drugs (Wlaź et al., 1996). This points to this therapeutic alternative as a more promising treatment for complex partial seizures in humans than the use of glycine$_B$ antagonists alone.

Acknowledgements

We wish to thank Dr. Wojcßiech Danysz (Merz + Co., Frankfurt/Main, Germany) for his comments on the manuscript. We would also like to thank Merck, Sharp and Dohme (Harlow, England) for a generous gift of L-701,324. This study was supported by Merz + Co. (Frankfurt/Main, Germany) and a research fellowship from the Alexander von Humboldt Foundation to Dr. P. Wlaź.

References

Baron, B.M., Harrison, B.L., Miller, F.P., McDonald, I.A., Salituro, F.G., Schmidt, C.J., Sorensen, S.M., White, H.S., Palfreyman, M.G., 1990. Activity of 5,7-dichlorokynurenic acid, a potent antagonist at the *N*-methyl-D-aspartate receptor-associated glycine binding site. Mol. Pharmacol. 38, 554–561.

Bristow, L.J., Flatman, K.L., Hutson, P.H., Kulagowski, J.J., Leeson, P.D., Young, L., Tricklebank, M.D., 1996a. The atypical neuroleptic profile of the glycine/*N*-methyl-D-aspartate receptor antagonist, L-701,324, in rodents. J. Pharmacol. Exp. Ther. 277, 578–585.

Bristow, L.J., Hutson, P.H., Kulagowski, J.J., Leeson, P.D., Matheson, S., Murray, F., Rathbone, D., Saywell, K.L., Thorn, L., Watt, A.P., Tricklebank, M.D., 1996b. The anticonvulsant and behavioural profile of L-701,324, a potent, orally active antagonist at the glycine modulatory site on the *N*-methyl-D-aspartate (NMDA) receptor complex. J. Pharmacol. Exp. Ther. 279, 492–501.

Carlson, H., Ronne-Engström, E., Ungerstedt, U., Hillered, L., 1992. Seizure related elevations of extracellular amino acids in human focal epilepsy. Neurosci. Lett. 140, 30–32.

Cotterell, K.L., Croucher, M.J., Bradford, H.F., 1992. Weak anticonvulsant activity of CGP 37849 and CGP 39551 against kindled seizures following systemic administration. Eur. J. Pharmacol. 214, 285–287.

Croucher, M.J., Bradford, H.F., 1990. 7-Chlorokynurenic acid, a strychnine-insensitive glycine receptor antagonist, inhibits limbic seizure kindling. Neurosci. Lett. 118, 29–32.

Croucher, M.J., Bradford, H.F., 1991. The influence of strychnine-insensitive glycine receptor agonists and antagonists on generalized seizure thresholds. Brain Res. 543, 91–96.

Croucher, M.J., Collins, J.F., Meldrum, B.S., 1982. Anticonvulsant action of excitatory amino acid antagonists. Science 216, 899–901.

Czuczwar, S.J., Meldrum, B., 1982. Protection against chemically induced seizures by 2-amino-7-phosphonoheptanoic acid. Eur. J. Pharmacol. 83, 335–338.

Danysz, W., Parsons, C.G., Karcz-Kubicha, M., Gold, M., Kalvinch, I., Piskunova, I., Rozhkov, E., 1996. Novel systemically active antagonists of the glycine site of the glycine site of NMDA receptor – behavioural characterisation. Soc. Neurosci. Abstr. 22, 1530.

Ebert, U., Rundfeldt, C., Löscher, W., 1995. Development and pharmacological suppression of secondary afterdischarges in the hippocampus of amygdala-kindled rats. Eur. J. Neurosci. 7, 732–741.

Ebert, U., Wlaź, P., Löscher, W., 1997. Anticonvulsant effects by combined treatment with a glycine$_B$ receptor antagonist and a polyamine site antagonist in amygdala-kindled rats. Eur. J. Pharmacol. 322, 179–184.

Globus, M.Y., Ginsberg, M.D., Busto, R., 1991. Excitotoxic index – a biochemical marker of selective vulnerability. Neurosci. Lett. 127, 39–42.

Ilyin, V.I., Whittemore, E.R., Tran, M., Shen, K.Z., Cai, S.X., Kher, S.M., Keana, J.F., Weber, E., Woodward, R.M., 1996. Pharmacology of ACEA-1416: A potent systemically active NMDA receptor glycine site antagonist. Eur. J. Pharmacol. 310, 107–114.

Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325, 529–531.

Juul-Jensen, P., 1986. Epidemiology of intractable epilepsy. In: Schmidt, D., Morselli, P. (Eds.), Intractable Epilepsy: Experimental and Clinical Aspects. Raven Press, New York, NY, pp. 5–11.

Kehne, J.H., Baron, B.M., Harrison, B.L., McCloskey, T.C., Palfreyman, M.G., Poirot, M., Salituro, F.G., Siegel, B.W., Slone, A.L., Van Giersbergen, P.L.M., White, H.S., 1995. MDL 100,458 and MDL 102,288: Two potent and selective glycine receptor antagonists with different functional profiles. Eur. J. Pharmacol. 284, 109–118.

Kito, S., Miyoshi, R., Nomoto, T., 1990. Influence of age on NMDA receptor complex in rat brain studied by in vitro autoradiography. J. Histochem. Cytochem. 38, 1725–1731.

Kleckner, N.W., Dingledine, R., 1988. Requirement for glycine in activation of NMDA-receptor expressed in *Xenopus* oocytes. Science 241, 835–837.

Kulagowski, J.J., Baker, R., Curtis, N.R., Leeson, P.D., Mawer, I.M., Moseley, A.M., Ridgill, M.P., Rowley, M., Stansfield, I., Foster, A.C., Grimwood, S., Hill, R.G., Kemp, J.A., Marshall, G.R., Saywell, K.L., Tricklebank, M.D., 1994. 3′-(Arylmethyl)- and 3′-(aryloxy)-3-phenyl-4-hydroxyquinolin-2(1H)-ones: Orally active antagonists of the glycine site on the NMDA receptor. J. Med. Chem. 37, 1402–1405.

Löscher, W., 1993. Basic aspects of epilepsy. Curr. Opin. Neurol. Neurosurg. 6, 223–232.

Löscher, W., Schmidt, D., 1988. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 2, 145–181.

Löscher, W., Hönack, D., 1991a. The novel competitive *N*-methyl-D-aspartate (NMDA) antagonist CGP 37849 preferentially induces phencyclidine-like behavioral effects in kindled rats: Attenuation by manipulation of dopamine, alpha-1 and serotonin$_{1A}$ receptors. J. Pharmacol. Exp. Ther. 257, 1146–1153.

Löscher, W., Hönack, D., 1991b. Anticonvulsant and behavioral effects of two novel competitive *N*-methyl-D-aspartic acid receptor antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK-801 and carbamazepine. J. Pharmacol. Exp. Ther. 256, 432–440.

Löscher, W., Nolting, B., Hönack, D., 1988. Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. Eur. J. Pharmacol. 152, 9–17.

Löscher, W., Horstermann, D., Hönack, D., Rundfeldt, C., Wahnschaffe, U., 1993. Transmitter amino acid levels in rat brain regions after amygdala-kindling or chronic electrode implantation without kindling: Evidence for a pro-kindling effect of prolonged electrode implantation. Neurochem. Res. 18, 775–781.

Löscher, W., Wlaź, P., Rundfeldt, C., Baran, H., Hönack, D., 1994. Anticonvulsant effects of the glycine/NMDA receptor ligands D-cycloserine and D-serine but not *R*-(+)-HA-966 in amygdala-kindled rats. Br. J. Pharmacol. 112, 97–106.

McNamara, J.O., Russell, R.D., Rigsbee, L., Bonhaus, D.W., 1988. Anticonvulsant and antiepileptogenic actions of MK-801 in the kindling and electroshock models. Neuropharmacology 27, 563–568.

Meldrum, B.S., Croucher, M.J., Badman, G., Collins, J.F., 1983. Antiepileptic action of excitatory amino acid antagonists in the photosensitive baboon *Papio papio*. Neurosci. Lett. 39, 101–104.

Namba, T., Morimoto, K., Yamada, N., Otsuki, S., 1993. Antiepileptogenic action of 7-chlorokynurenic acid on amygdala-kindling of rats. Pharmacol. Biochem. Behav. 46, 275–281.

Parsons, C.G., Danysz, W., Quack, G., Hartmann, S., Lorenz, B., Baran, L., Przegaliński, E., Kostowski, W., Krzascik, P., Headley, P.M., Chizh, B., 1997. Novel systemically-active antagonists of the glycine site of the NMDA receptor – electrophysiological, biochemical and behavioral characterisation. J. Pharmacol. Exp. Ther., in press.

Patel, S., Chapman, A.G., Graham, J.L., Meldrum, B.S., Frey, P., 1990. Anticonvulsant activity of the NMDA antagonists, D(−)4-(3-phosphonopropyl) piperazine-2-carboxylic acid (D-CPP) and D(−)(E)-4-(3-phosphonoprop-2-enyl) piperazine-2-carboxylic acid (D-CPPene) in a rodent and a primate model of reflex epilepsy. Epilepsy Res. 7, 3–10.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, Sydney.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Rogawski, M.A., 1995. Excitatory amino acids and seizures. In: Stone, T.W. (Ed.), CNS Neurotransmitters and Neuromodulators: Glutamate. CRC Press, Boca Raton, FL, pp. 219–237.

Ronne-Engström, E., Hillered, L., Flink, R., Spannare, B., Ungerstedt, U., Carlson, H., 1992. Intracerebral microdialysis of extracellular amino

acids in the human epileptic focus. J. Cereb. Blood Flow Metab. 12, 873–876.

Rowley, M., Leeson, P.D., Stevenson, G.I., Moseley, A.M., Stansfield, I., Sanderson, I., Robinson, L., Baker, R., Kemp, J.A., Marshall, G.R., Foster, A.C., Grimwood, S., Tricklebank, M.D., Saywell, K.L., 1993. 3-Acyl-4-hydroxyquinolin-2(1H)-ones. Systemically active anticonvulsants acting by antagonism at the glycine site of the *N*-methyl-D-aspartate receptor complex. J. Med. Chem. 36, 3386–3396.

Rundfeldt, C., Wlaź, P., Löscher, W., 1994. Anticonvulsant activity of antagonists and partial agonists for the NMDA receptor-associated glycine site in the kindling model of epilepsy. Brain Res. 653, 125–130.

Scatton, B., 1993. The NMDA receptor complex. Fundam. Clin. Pharmacol. 7, 389–400.

Schmutz, M., Portet, C., Jeker, A., Klebs, K., Vassout, A., Allgeier, H., Heckendorn, R., Fagg, G.E., Olpe, H.R., van Riezen, H., 1990. The competitive NMDA receptor antagonists CGP 37849 and CGP 39551 are potent, orally-active anticonvulsants in rodents. Naunyn–Schmiedebergs Arch. Pharmacol. 342, 61–66.

Smith, S.E., al Zubaidy, Z.A., Chapman, A.G., Meldrum, B.S., 1993. Excitatory amino acid antagonists, lamotrigine and BW 1003C87 as anticonvulsants in the genetically epilepsy-prone rat. Epilepsy Res. 15, 101–111.

Sveinbjornsdottir, S., Sander, J.W., Upton, D., Thompson, P.J., Patsalos, P.N., Hirt, D., Emre, M., Lowe, D., Duncan, J.S., 1993. The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy. Epilepsy Res. 16, 165–174.

Tortella, F.C., Hill, R.G., 1996. EEG seizure activity and behavioral neurotoxicity produced by (+)-MK801, but not the glycine site antagonist L-687,414, in the rat. Neuropharmacology 35, 441–448.

Tricklebank, M.D., Bristow, L.J., Hutson, P.H., Leeson, P.D., Rowley, M., Saywell, K., Singh, L., Tattersall, F.D., Thorn, L., Williams, B.J., 1994. The anticonvulsant and behavioural profile of L-687,414, a partial agonist acting at the glycine modulatory site on the *N*-methyl-D-aspartate (NMDA) receptor complex. Br. J. Pharmacol. 113, 729–736.

Witkin, J.M., Steele, T.D., Sharpe, L.G., 1997. Effects of strychnine-insensitive glycine receptor ligands in rats discriminating dizocilpine or phencyclidine from saline. J. Pharmacol. Exp. Ther. 280, 46–52.

Wlaź, P., Baran, H., Löscher, W., 1994a. Effect of the glycine/NMDA receptor partial agonist, D-cycloserine, on seizure threshold and some pharmacodynamic effects of MK-801 in mice. Eur. J. Pharmacol. 257, 217–225.

Wlaź, P., Ebert, U., Löscher, W., 1994b. Low doses of the glycine/NMDA receptor antagonist *R*-(+)-HA-966 but not D-cycloserine induce paroxysmal activity in limbic brain regions of kindled rats. Eur. J. Neurosci. 6, 1710–1719.

Wlaź, P., Roliński, Z., Czuczwar, S.J., 1996. Influence of D-cycloserine on the anticonvulsant activity of phenytoin and carbamazepine against electroconvulsions in mice. Epilepsia 37, 610–617.