The Role of Probenecid-Sensitive Organic Acid Transport in the Pharmacokinetics of N-Methyl-D-Aspartate Receptor Antagonists Acting at the Glycine<sub>B</sub>-Site: Microdialysis and Maximum Electroshock Seizures Studies


Department of Pharmacological Research (M.B.H., H.S., B.E., W.D.), Merz + Co., Frankfurt/Main, Germany

Accepted for publication April 23, 1999

ABSTRACT

The purpose of the present study was to determine whether the probenecid-sensitive organic acid transporter is responsible for the short duration of action of a new group of N-methyl-D-aspartate receptor glycine<sub>B</sub>-site antagonists, MRZ 2/570, 2/571, and 2/576. A prolongation of their anticonvulsant activity from 60 to 180 to 240 min, was found in mice after pretreatment with probenecid (200 mg/kg i.p.). Microdialysis studies in rats showed that this is likely due to a change in the central nervous system concentrations of these drugs because cotreatment with probenecid caused an increase in the brain extracellular fluid half-life (0.5- to 4-fold) and the brain area under the curve (1.8- to 3.6-fold). In serum the half-life of MRZ 2/576 (30 mg/kg) was also increased by coadministration of probenecid from 15.6 ± 1.3 to 40.6 ± 6.0 min. At steady state (MRZ 2/576, 20 mg/kg/h i.v.), brain extracellular fluid concentration was elevated 2.5-fold by concomitant administration of probenecid. These results clearly show that these glycine<sub>B</sub>-site antagonists are rapidly cleared from the systemic circulation and the central nervous system by the probenecid-sensitive organic acid transport system. Moreover, the present data show that MRZ 2/570, 2/571, and 2/576 reach the brain in concentrations (1.34–2.32 μM) above the range of their in vitro potencies at the glycine<sub>B</sub> site of the N-methyl-D-aspartate receptor (0.1–1.0 μM).

The glutamatergic N-methyl-D-aspartate (NMDA) receptor complex consists of several distinct binding domains that allow modulation of NMDA receptor-mediated responses. One of them that has attracted considerable attention in recent years is the strychnine-insensitive glycine<sub>B</sub>-site antagonists, which have been focused on glycine<sub>B</sub>-site antagonists, which have been suggested to be apparently devoid of these side effects and seem to have a more favorable therapeutic profile (Bristow et al., 1996; Kretschmer et al., 1997; Danysz and Parsons, 1998). However, with competitive and uncompetitive NMDA antagonists some side effects such as PCP-like psychotomimetic effects and vacuolization of the cingulate/retrosplenial cortex are known to occur at high doses (see Parsons et al., 1998). Thus recently more attention has focused on glycine<sub>B</sub>-site antagonists, which have been suggested to be apparently devoid of these side effects and seem to have a more favorable therapeutic profile (Bristow et al., 1996; Kretschmer et al., 1997; Danysz and Parsons, 1998; Parsons et al., 1998).

To date the major problem with the use of glycine<sub>B</sub>-site antagonists has been their poor bioavailability. However, recently some full antagonists of the glycine<sub>B</sub>-site with better brain penetration have become available (see Parsons et al., 1998; Danysz and Parsons, 1998). Efficacy after systemic administration has been shown in models of epilepsy (MDL 104,653; Chapman et al., 1995), hyperalgesia (ACEA-1011; Vaccarino et al., 1993), focal ischemia (7-chlorothiokynurenec

Received for publication October 7, 1998.

† Current address: Department of Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2300 RA Leiden, the Netherlands.

‡ On leave from Department of Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2300 RA Leiden, the Netherlands.

§ Present address: Department of Neuropharmacology, Cerebrus Ltd., Oakdene Court, 613 Reading Road, Winnersh, Wokingham, RG41 6UA, UK.

© Present address: Department of Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2300 RA Leiden, the Netherlands.

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; CNS, central nervous system; ECF, extracellular fluid; AUC, area under the curve; glycine<sub>B</sub>-site, strychnine-insensitive glycine coagonist site; BBB, blood-brain barrier; MES, maximal electroshock; aCSF, artificial cerebrospinal fluid; PNQX, pyrido[3,4-f]quinoline-2,3-dione,1,4,7,8,9,10-hexahydrop-9-methyl-6-nitromethanesulfonate.
applied through corneal electrodes. The presence of tonic convulsions Afterwards, MES (100 Hz, 0.5-s shock duration, 50 mA shock intensity of 30 mg/kg), L-701,324 (6 mg/kg), or memantine (20 mg/kg) were used for MES-induced convulsions and male Sprague-Dawley rats (235–270 g) were used for microdialysis experiments. All animals used for the probenecid-sensitive organic acid transport in their clearance. The most likely carrier system responsible for this phenomenon is the organic acid transport system located in the epithelium of both the choroid plexus and kidney and possibly at the endothelium of the blood-brain barrier (BBB; Adkinson et al., 1994; Deuguchi et al., 1995). It transports unselectively xenobiotics with an organic acid structure at physiological pH out of the cerebrospinal fluid into the bloodstream and out of the systemic circulation into the urine. This carrier can be competitively inhibited by probenecid, which was developed in the 1950s to prolong the duration of action of antibiotics by inhibition of renal clearance. Lately attention has been focused again on the effect of probenecid on the pharmacokinetics of a number of different classes of drug with short durations of action, such as AZT (Dykstra et al., 1993; Wang and Sawchuck, 1995), valproic acid (Golden et al., 1993; Naora et al., 1996), and both β-lactam and quinolone antibiotics (Suzuki et al., 1997). Also the CNS actions of NMDA receptor antagonists such as kynurenic acid and quinoxaline derivatives have been shown to be prolonged by coadministration of probenecid (Taylor and Vartanian, 1992; Santa-Maria et al., 1996; Danysz and Parsons, 1998).

We have recently developed a series of tricyclic pyrido-pthalazine-diones, which are active in a number of animal models after systemic administration, indicating sufficient brain penetration, but their duration of action is rather short (Parsons et al., 1997; Popik et al., 1998; Wenk et al., 1998; Karcz-Kubička et al., 1999). Hence, this study focused on the CNS pharmacokinetic profile of these agents and the role of the probenecid-sensitive organic acid transport in their clearance. CNS presence was determined directly using brain microdialysis in rats and indirectly by assessing the anticonvulsant effect of probenecid in maximal electroshock (MES)-induced convulsions in mice. Some of these results were presented already in abstract form (Hesselink et al., 1997).

Materials and Methods

Subjects

Female Naval Medical Research Institute mice (18–28 g) were used for MES-induced convulsions and male Sprague-Dawley rats (235–270 g) were used for microdialysis experiments. All animals were obtained from Charles River (Sulzfeld, Germany). The rats were housed 5 per cage and the mice 15 per cage. During recovery from microdialysis surgery the rats were housed separately. All animals were kept under standard laboratory conditions: 12-h light/dark cycle, 20°C, and free access to food and water.

MES-Induced Convulsions

MRZ 2/570 (30 mg/kg), MRZ 2/571 (30 mg/kg), MRZ 2/576 (20 and 30 mg/kg), L-701,324 (6 mg/kg), or memantine (20 mg/kg) were injected i.p. 30 min after probenecid (200 mg/kg) or vehicle (also i.p.). Afterwards, MES (100 Hz, 0.5-s shock duration, 50 mA shock intensity, 0.9-ms impulse duration, Ugo Basile, Basel, Switzerland) was applied through corneal electrodes. The presence of tonic convulsions was scored (tonic extension of hind paws with minimum angle to the body of 90°).

Brain Microdialysis

Microdialysis Surgery. The rats were anesthetized with Hypnorm (1.0 ml/kg i.m.; Janssen-Cilag, Buckinghamshire, UK), placed in a stereotaxic frame, and the skull was exposed. A small hole was drilled to allow the implantation of a microdialysis guide cannula (CMA/10, CMA Microdialysis, Solna, Sweden) in the anterior striatum relative to bregma (anterior/posterior: 1.0; lateral: 2.5; vertical: −3.0). A second hole was drilled, a screw was secured into the skull, and the screw and guide cannula were cemented together onto the skull using dental cement (Paladur, Heraeus, Germany). A microdialysis probe (CMA/10, membrane length of 3.0 mm) was inserted into the guide cannula immediately after surgery and the animals were allowed to recover for 22 to 26 h. In the animals that received MRZ 2/576 as an i.v. infusion, the femoral vein was cannulated immediately after guide cannula implantation.

Microdialysis Experiment. At the start of the experiment the inflow tubing was connected to a syringe pump (CMA/100, syringe pump) by means of a dual channel swivel (CMA) and the probe was perfused with artificial cerebrospinal fluid (aCSF, composition in mM: NaCl, 145; KCl, 0.6; MgCl₂, 1.0; CaCl₂, 1.2; ascorbic acid, 0.2, in a 2 mM potassium phosphate buffer, pH 7.4) at a flow rate of 3 μl/min. The outlet tubing was connected to a microfraction collector (CMA/140) and 10-min fractions were collected. After 2 h of dialysis MRZ 2/570, 2/571, 2/576 (30 mg/kg or 10–40 mg/kg for MRZ 2/576), or memantine (20 mg/kg) was injected i.p. Microdialysate samples were collected for up to 180 min for the glycine_1-antagonists and 240 min for memantine. Probenecid (200 mg/kg) was administered 30 min before the administration of the glycine_1 antagonists or memantine.

In Vitro Recovery. A microdialysis probe (CMA 10, membrane length 3 mm) was placed in a stirred aCSF solution maintained at 37°C and containing 1 μM MRZ 2/570, 2/571, 2/576, or memantine. The probe was perfused (CMA/100, syringe pump) with aCSF at a speed of 3 μl/min for 2 h to allow establishment of steady state before three 20-min samples were collected.

In Vivo Zero-Net Flux Method. The animals were connected to an infusion pump (WPI, SP100i; World Precision Instruments, Sarasota, FL) 2 h before perfusion of the microdialysis probe started for steady-state plasma concentrations to develop. MRZ 2/576 was infused at a dose of 20 mg/kg/h i.v. Subsequently, the microdialysis probe was continuously perfused with aCSF containing different concentrations MRZ 2/576 (four different concentrations ranging from 0–5 μM). Every inflow concentration was perfused for 2 h before three 20-min samples were collected.

Serum Pharmacokinetics

Canulla Implantation. For serum sampling the femoral artery was cannulated using silicone tubing (i.d. 0.508, o.d. 0.9398) under Nembutal (Sanoﬁ, Hannover, Germany) anesthesia. The cannula was led subcutaneously to the neck of the animal, where a small incision was made in the skin. The outlet of the cannula was secured using a small rodent jacket (Lomir Biomedical, Malone, NY). The animals were allowed to recover for 20 to 24 h before the experiment started.

Serum Sample Collection. A 30-cm-long polyethylene tubing, filled with a heparin solution (500 U/ml), was connected to the cannula outlet. The animals were placed in a plastic rat bowl (CMA) separately and were allowed to move freely during the experiment. MRZ 2/570, 2/571, or 2/576 was injected i.p. at a dose of 30 mg/kg and 250-μl samples were taken after 2, 5, 10, 20, 40, 60, and 90 min. When probenecid (200 mg/kg i.p.) was given 30 min before administration of the glycine_1-antagonists, serum samples were also taken after 120 and 150 min. Memantine (20 mg/kg) was given i.p. and samples were taken after 15, 30, 60, 120, 150, 240, 300, 360, and 480 min.
min. Aliquots (50 μl) of serum were pipetted into 2-ml screw-capped test tubes. After addition of 500 μl trifluoroacetic acid solution (0.1%) the samples were mixed for 1 min on a vortex mixer and 1 h on a BC1 mixer. After centrifugation for 10 min the sample was directly injected on the HPLC system (column switching). The memantine samples were analyzed using gas chromatography as described previously (Hesselink et al., 1999).

Substances

The following compounds were used: MRZ 2/570, MRZ 2/571, and MRZ 2/576 (8-Br-, 8-F, and 8-Cl-4-hydroxy-1-oxo-1,2-dihydropyridalino(4,5-b)quinoline-5-oxide choline salt, Merz + Co., Frankfurt/Main, Germany), L-701,324 (7-chloro-4-hydroxy-3-(-phenoxy)-phenyl-2Hquinolone; Tocris Cookson, Bristol, UK), memantine (HCl, 1-amino-3,5-dimethyladamantine, Merz + Co), and probenecid (Sigma, Deisenhofen, Germany). Probenecid was suspended in 0.5% CMC (carboxymethylcellulose) with 0.1% Tween 80 in water. MRZ 2/570, 2/571, and 2/576 were dissolved in water and memantine was dissolved in saline. L-701,324 was suspended in 25% polyethylene glycol and adjusted with 1 N NaOH to pH 8.0. All compounds were dissolved at a concentration to assure a injection volume of 1 ml/kg for rats i.p. or i.v. and 10 ml/kg for mice i.p.

Results

MES-Induced Convulsions. To investigate a functional interaction between the tested glycineB-site antagonists and probenecid the duration of anticonvulsive activity of MRZ 2/570, 2/571, and 2/576 was measured with and without poadministration of probenecid. Previously it has been shown that this dose of probenecid (200 mg/kg i.p.) does not have an effect on MES-induced convulsions on its own (Taylor and Vartanian, 1992; Parsons et al., 1997). MRZ 2/576 at doses of 20 and 30 mg/kg i.p. protected against MES-induced convulsions (Fig. 1, A and B) with a short duration of action,

![Fig. 1. Probenecid prolongs the anticonvulsant action of the glycineB-site NMDA antagonists: MRZ 2/576 (A and B), MRZ 2/570 (C), MRZ 2/571 (D), and L-701,324 (E) but not of the uncompetitive NMDA antagonist memantine (F) in the MES model in mice. Probenecid (200 mg/kg i.p.) was given 30 min before administration of the test compounds. MRZ 2/576 was given at a dose of 20 (A) and 30 (B) mg/kg i.p. MRZ 2/570, MRZ 2/571, L701,324, and memantine were given at doses of 30, 40, 6, and 20 mg/kg, respectively. Data are expressed as mean ± S.E.M. (n = 4–6).](image-url)
i.e., less than 60 min. When probenecid (200 mg/kg i.p.) was administered 30 min before MRZ 2/576, a prolongation of the anticonvulsive activity to 180 to 240 min was seen. A similar effect of probenecid was observed with the other NMDA receptor glycineB-site antagonists MRZ 2/570, MRZ 2/571, and L-701,324 (Fig. 1, C–E). In case of the channel blocker memantine, only a minor difference between the probenecid-pretreated animals and nontreated animals existed 6 h after injection of memantine, i.e., 6.5 h after injection of probenecid (Fig. 1F). This was probably not directly related to probenecid treatment because its effect starts to decrease already after 3 h as shown by the experiment with glycineB-site antagonist MRZ 2/576 (Fig. 6), hence after 6 h its action should be marginal.

**In Vitro and In Vivo Recovery.** The three glycineB-site antagonists, tested, i.e., MRZ 2/570, 2/571, and 2/576, showed similar in vitro recovery (15.5 ± 4.7, 17.9 ± 2.4, and 12.9 ± 3.2%, respectively). For memantine an in vitro recovery of 18.0 ± 4.8% was obtained. Figure 2 shows the in vivo recovery of MRZ 2/576 using the zero-net flux method for four separate animals. Using this method, the in vivo recovery can be estimated by taking the inverse of the slope of the linear regression line when the difference between ingoing and outgoing dialysate concentration (C_{out} - C_{in}) is plotted against the ingoing concentration (C_{in}). For MRZ 2/576 an in vivo recovery of 32 ± 5% was found. All subsequent microdialysate concentrations of MRZ 2/570, 2/571, and 2/576 were corrected for this in vivo recovery. The in vivo recovery of memantine (39 ± 2%) was determined previously (Hesselink et al., 1999).

**Microdialysis Studies after Acute Injection of MRZ 2/570, 2/571, 2/576, or Memantine–Interaction with Probencid.** Microdialysis studies were performed to determine the brain pharmacokinetics of the selected glycineB-site antagonists. After administration of 30 mg/kg i.p. the peak concentration of the glycineB-site antagonists in striatal extracellular fluid (ECF) ranged from 1.34 to 2.32 μM (MRZ 2/576 and 2/571, respectively; Fig. 3, A–C). The short duration of their anticonvulsive activity in the MES-induced convulsion model in mice corresponds to short brain ECF half-life ranging from 14.7 min for MRZ 2/571 to 20.2 min for MRZ 2/576 (Table 1). To evaluate whether the interaction between probenecid and glycineB-site antagonists in the MES test was of a pharmacokinetic nature, microdialysis studies were performed with probenecid (200 mg/kg). Pretreatment with probenecid increased the brain ECF half-life and area under the curve (AUC; 1.8- to 3.6-fold) for all three glycineB-site antagonists (Fig. 3, A–C; Table 1). Some variation was observed...
between the tested agents in the effect of probenecid on their brain ECF half-life (Table 1). For MRZ 2/576, only a 50% increase was apparent, from 20.2 to 31.2 min, whereas in the case of MRZ 2/570 its half-life increased 4-fold (from 14.7 to 57.7 min). A difference between the three compounds was also apparent in a delay and magnitude of peak concentration (Fig. 3, A–C). Administration of probenecid did not affect the peak concentration of either MRZ 2/570 or 2/571. In the case of MRZ 2/576 however, pretreatment with probenecid caused a 2-fold increase the peak concentration reached in the striatal ECF (Fig. 3C).

The effect of probenecid on the pharmacokinetics of the NMDA receptor-channel blocker memantine served as a negative control to ascertain that the interaction seen for the glycineB-site antagonists is a specific one for this class of compounds. Memantine is an uncompetitive antagonist of the NMDA receptor, which binds to a different site on the NMDA receptor than the glycineB-site antagonists (Parsons et al., 1999). Memantine is structurally unrelated to MRZ 2/570, MRZ 2/571, and MRZ 2/576 and shows high lipophilicity and very good penetration to the brain (Hesselink et al., 1999). It does, however, have a comparable effect on MES-induced convulsions in mice. Hence, it was considered an appropriate control to determine whether the interaction with probenecid is specific for the glycineB-site antagonists or whether it occurred with all antagonists of the NMDA receptor. Pretreatment with probenecid did not affect the concentration of memantine in striatal ECF (Fig. 4A).

Serum Pharmacokinetics of MRZ 2/576 and Memantine–Interaction with Probenecid. Serum concentrations of MRZ 2/576 were considerably higher than brain ECF concentrations. The peak concentration in serum was 94.4 μM (Fig. 5A) whereas in the brain ECF only 1.34 μM was reached. Thus by comparing the AUCs in serum and ECF a brain penetration of 2% can be calculated. Coadministration of probenecid increased the AUC of MRZ 2/576 in serum 1.6-fold and in ECF 3.6-fold (Table 1), enhancing brain ECF versus serum ratio to 4%. The effect of probenecid on the serum elimination half-life was more pronounced, i.e., it increased from 15.6 to 40.6 min by pretreatment with probenecid (Table 1).

Serum concentrations of the NMDA receptor channel blocker memantine were not affected by pretreatment with probenecid (Fig. 4B).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC (μg · h/ml)</th>
<th>T½ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRZ 2/570</td>
<td>0.217 ± 0.040</td>
<td>14.7 ± 1.7</td>
</tr>
<tr>
<td>MRZ 2/570 + probenecid</td>
<td>0.666 ± 0.107</td>
<td>57.7 ± 16.2</td>
</tr>
<tr>
<td>MRZ 2/571</td>
<td>0.496 ± 0.074</td>
<td>19.7 ± 1.1</td>
</tr>
<tr>
<td>MRZ 2/571 + probenecid</td>
<td>0.869 ± 0.110</td>
<td>39.8 ± 4.1</td>
</tr>
<tr>
<td>MRZ 2/576</td>
<td>0.188 ± 0.076</td>
<td>20.2 ± 0.2</td>
</tr>
<tr>
<td>MRZ 2/576 + probenecid</td>
<td>0.671 ± 0.075</td>
<td>31.2 ± 2.5</td>
</tr>
<tr>
<td>Serum: MRZ 2/576</td>
<td>9.7 ± 0.6</td>
<td>15.6 ± 1.3</td>
</tr>
<tr>
<td>Serum: MRZ 2/576 + probenecid</td>
<td>16.6 ± 1.7</td>
<td>40.6 ± 6.0</td>
</tr>
</tbody>
</table>

Fig. 4. Coadministration of probenecid does not effect the brain ECF (A) and plasma pharmacokinetics (B) of the uncompetitive NMDA antagonist memantine. Probenecid (200 mg/kg i.p.) was given 30 min before the administration of 20 mg/kg of memantine. Data are expressed as mean ± S.E.M. (n = 5).

Fig. 5. The effect of coadministration of probenecid on the serum pharmacokinetics of the glycineB-site NMDA antagonist MRZ 2/576. Probenecid (200 mg/kg i.p.) was given 30 min before the administration of 30 mg/kg of the glycineB-site antagonists. Data are expressed as mean ± S.E.M. (n = 4).

Microdialysis Studies after Continuous i.v. Infusion of MRZ 2/576–Interaction with Probenecid. The concentration of MRZ 2/576 in the striatal ECF was measured under steady-state conditions achieved through continuous i.v. infusion (20 mg/kg/h). Probenecid was subsequently administered systemically and changes in brain ECF concentrations were determined (Fig. 6). Before administration of
probenecid a steady-state concentration of MRZ 2/576 of 0.174 ± 0.015 μM was found, which was not significantly different between four control measurements. Administration of probenecid (200 mg/kg i.p.) caused a 2.5-fold increase in brain ECF concentration (Fig. 6). A clear effect of probenecid could be seen 40 to 200 min after its administration.

**Dose Dependence of Brain Pharmacokinetics of MRZ 2/576.** If MRZ 2/576 is transported out of the CNS by an active transport system, this transport system could putatively be saturated by higher concentrations of MRZ 2/576 itself. To determine whether this is the case, increasing doses of MRZ 2/576 (10–40 mg/kg i.p.) were administered and the brain ECF concentration was measured. A nonlinear relationship exists between the dose administered and the brain ECF concentration of MRZ 2/576 (Fig. 7). The AUC found after administration of 10 to 30 mg/kg MRZ 2/576 ranged from 0.071 ± 0.019 to 0.188 ± 0.076 μg·h/ml for 10 and 30 mg/kg i.p., respectively, whereas after 40 mg/kg of MRZ 2/576 considerably higher levels were found i.e., an AUC of 0.484 ± 0.060 μg·h/ml.

**Discussion**

The present results clearly show a functional potentiation of duration of anticonvulsive action of glycineB-site antagonists (MRZ 2/570, MRZ 2571, MRZ 2/576, and L-701,324) against MES seizures when probenecid was preadministered. Such an interaction was not observed with the NMDA channel blocker memantine. Based on the microdialysis data it can be concluded that this interaction is most likely pharmacokinetic in nature because the brain ECF half-lives of the glycineB-site antagonists were increased by probenecid from 0.5- to 4-fold.

The data presented are the first direct proof that after systemic administration of 30 mg/kg the tested glycineB antagonists reached concentrations in the brain ECF (corrected for the in vivo recovery) high enough to block NMDA receptor-mediated neurotransmission (see Parsons et al., 1997). It was reported previously that in patch-clamp experiments, steady-state inward current responses of cultured hippocampal neurons to NMDA (200 μM, glycine 1 μM) were antagonized by MRZ 2/570, 2/571, and 2/576 with IC50 values of 0.14, 1.02, and 0.54 μM, respectively (Parsons et al., 1997). These concentration are exceeded in the brain ECF for a duration of ca. 60 min for all three compounds (see Fig. 4, A–C). This is in line with present MES-induced convulsion studies because mice were protected from MES-induced convulsions when the concentrations of MRZ 2/570, 2/571, and 2/576 in the brain ECF were high enough to block NMDA receptor-mediated transmission. Of course differences between species, rats versus mice, should also be taken into account. Previous studies demonstrated the efficacy of these compounds in animal tests related to opioid dependence (Popik et al., 1998), parkinsonism (Karcz-Kubicha et al., 1999), and neuroprotection (Wenk et al., 1998). However in the case of MRZ 2/576, when brain ECF concentrations are compared to serum levels (AUCs) it becomes clear that only 2% brain penetration is achieved.

Probenecid effect in the MES model seems to be specific because the anticonvulsant activity as well as pharmacokinetics of the channel-blocker memantine (negative control) were not changed significantly. Moreover, the pharmacokinetic data support the hypothesis that the interaction between probenecid and the glycineB-site antagonists is in fact a pharmacokinetic one because pretreatment with probenecid increased the brain ECF half-life and the brain AUCs of the glycineB-site antagonists. Taken together, this indicates that the elimination of MRZ 2/570, 2/571, and 2/576 from the CNS and the systemic circulation involves the probenecid-sensitive organic acid transporter. Because this transporter is also located at the epithelium of the kidney, the effect of probenecid on the serum pharmacokinetics of these compounds was determined to ascertain whether an effect of probenecid in the kidney could solely explain the increased brain concentrations. The AUC of MRZ 2/576 in serum was...
increased 1.7-fold, whereas for the brain ECF the AUC was increased 3.6-fold, which would indicate that there is an additive effect of the transporters located in the choroid plexus, BBB, and kidney. However, the effect on elimination out of the brain seems to predominate. Moreover, a possible interaction of probenecid and the glycine_receptor antagonists could arise at the level of plasma protein binding. Probenecid binds strongly to plasma protein and might then through reduction of the protein binding of the glycine_receptor antagonists increase the free fraction. This might be particularly true for MRZ 2/576 because peak levels in the brain were increased by probenecid. However, it has previously been determined that warfarin, which is highly plasma protein-bound, has only a minor effect on MRZ 2/576 anticonvulsant effects in MES-induced convulsions in mice (Parsons et al., 1997). Moreover, preliminary experiments indicated that warfarin did not change brain pharmacokinetics of MRZ 2/570 (M.B.H. and W.D., not published). Moreover, displacing tested glycine antagonists from plasma proteins should rather result in shortening than prolonging the half-lives.

Because the probenecid-sensitive organic acid transporter is saturable it is likely that these compounds could saturate this carrier system on their own at higher doses. If this holds true their brain ECF concentration would be disproportionately increased at high doses. This can in fact be observed with MRZ 2/576 at doses that exceed 30 mg/kg. On the basis of these results this cannot be exclusively attributed to reduced transport out of the brain and might be explained by a decrease in clearance from the blood by a saturation of the transporter in the kidney or a combination of both.

First indications that compounds acting on glutamate receptors are transported out of the CNS by the probenecid-sensitive organic acid transporter came from studies with kynurenic acid (Moroni et al., 1988). Systemic administration of probenecid increases endogenous levels of either endogenous kynurenic acid or that provided by its systemically available precursor L-kynurenine (Miller et al., 1992). Moreover, preliminary experiments indicated that probenecid did not change brain pharmacokinetics of MRZ 2/570 (M.B.H. and W.D., not published). Moreover, displacing tested glycine antagonists from plasma proteins should rather result in shortening than prolonging the half-lives.

Because the probenecid-sensitive organic acid transporter is saturable it is likely that these compounds could saturate this carrier system on their own at higher doses. If this holds true their brain ECF concentration would be disproportionately increased at high doses. This can in fact be observed with MRZ 2/576 at doses that exceed 30 mg/kg. On the basis of these results this cannot be exclusively attributed to reduced transport out of the brain and might be explained by a decrease in clearance from the blood by a saturation of the transporter in the kidney or a combination of both.

First indications that compounds acting on glutamate receptors are transported out of the CNS by the probenecid-sensitive organic acid transporter came from studies with kynurenic acid (Moroni et al., 1988). Systemic administration of probenecid increases endogenous levels of either endogenous kynurenic acid or that provided by its systemically available precursor L-kynurenine (Miller et al., 1992). This approach provided protection against NMDA, quinolinic acid, available precursor L-kynurenine (Miller et al., 1992). This nonspecific action of kynurenic acid or that provided by its systemically administered L-kynurenine (Moroni et al., 1988). Systemic administration of probenecid, whereas the PNQX concentration in the brain ECF was increased 2.5-fold after administration (Jordan et al., 1997). This indicates that not only the clearance from the brain contributes to PNQX's short brain half-life, but also clearance from the systemic circulation probably by the probenecid-sensitive organic acid transporter located on the kidney epithelium.

The present results obtained using i.v. infusion of MRZ 2/576 are comparable with the previously mentioned data with PNQX (Jordan et al., 1997). In the case of MRZ 2/576 the concentration in the brain ECF was increased 2.5-fold after administration of probenecid, whereas the PNQX concentration increased 4-fold. However, in the present study probenecid was administered as a single i.p. injection, whereas in combination with PNQX probenecid was continuously infused, apparently leading to more efficient transporter inhibition due to a higher probenecid concentration in the brain (Jordan et al., 1997). Moreover, these differences might also be explained by different transport affinities of the glycine_receptor-sensitive organic acid transporter for PNQX, MRZ 2/576, and probenecid.

One of the major challenges to tackle when using microdialysis in pharmacokinetic studies is the issue of recovery, i.e., relating dialysate to brain ECF concentrations. The recovery, also called extraction fraction, is defined as the ratio between medium (here brain ECF) and dialysate concentrations. Several methods for determining the recovery have been developed, ranging from the simplest in vitro method to the more complicated in vivo zero-net flux and mass-transfer methods (Benveniste and Hüttemeier, 1990; Bungay et al., 1990). For the glycine_receptor antagonists comparable in vitro recoveries were found for all three compounds. They all have a pyrido[3,4-d]pyridazine structure but are substituted by different halogens at position 8: bromo-, fluoro-, and chloro- for MRZ 2/570, 2/571, and 2/576, respectively (Parsons et al., 1997). With the homology in chemical structure one could assume the same or comparable physicochemical properties and diffusion kinetics.

Surprising was the observation that MRZ 2/576 had a higher in vivo than in vitro recovery. The recovery of a compound is determined by its diffusion kinetics, the perfusion flow rate, the microdialysis membrane characteristics, and the surrounding medium; in the case of in vitro recovery, an artificial CSF solution (Benveniste and Hüttemeier, 1990). In general it has been found that for hydrophilic compounds the in vivo recovery is lower than the in vitro recovery. This is due to the complex pattern of diffusion in the brain, the gluttinous character of the extracellular matrix, and the fact that diffusion is limited to the ECF (Benveniste et al., 1989). However, exceptions to this have been noted for compounds that are actively transported. Menacherry and coworkers (1992), explained the fact that a higher in vivo than in vitro recovery was found for cocaine as an effect of microvasculature transport, i.e., BBB transport. The concentration gradient of cocaine in close vicinity of the probe is dramatically changed when BBB transport is taken into account (Morrison et al., 1991). This phenomenon of disturbance in concentration gradient of a solute in close vicinity of the probe as a result of removal of solute from the tissue by transport or metabolism, was first described by Bungay and colleagues (1990) for the reference compounds [3H]H2O, sucrose, and 3,4-dihydroxyphenylacetic acid. If MRZ 2/576 is in the fact transported by the organic acid transporter, this might cause a change in the concentration gradient in close vicinity of the microdialysis probe. When the depletion zone in close vicinity of the probe is replenished by a gradient of MRZ 2/576 created by active transport, a steeper concentration gradient is created, which in turn leads to a higher probe recovery. The presented data once more point out the importance of calibrating the microdialysis probe using in vivo as well as in vitro recovery, because comparing the two offers a better understanding of the diffusion kinetics in close vicinity of the microdialysis probe.

The present study provides the first direct proof that MRZ
glycineB antagonist at the doses effective in a number of animal models reach the brain ECF at concentrations sufficient to influence NMDA receptor-mediated transmission. Moreover, it also clearly shows that the glycineB-site antagonists are rapidly cleared from the CNS and the systemic circulation by the probenecid-sensitive organic acid transport system, i.e., their short duration of action can be prolonged by pretreatment with probenecid and this cotreatment approach could be a valid therapeutic strategy in the future.

Acknowledgments

We thank Bianka Lorenz for her assistance with the MES experiments.

References


Send reprint requests to: Dr. Wojciech Danyus, Merz + Co., Dept. of Pharmacological Research, Eckenheimer Landstrasse 100–104, 60318 Frankfurt/Main, Germany. E-mail: wojciech.danyus@merz.de