Quantitative in Vivo Microdialysis Study on the Influence of Multidrug Transporters on the Blood-Brain Barrier Passage of Oxcarbazepine: Concomitant Use of Hippocampal Monoamines as Pharmacodynamic Markers for the Anticonvulsant Activity

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ABSTRACT

Various antiepileptic drugs were shown to be substrates for multidrug transporters at the level of the blood-brain barrier. These ATP-dependent efflux pumps actively limit brain accumulation of xenobiotics and drugs. Intrahippocampal oxcarbazepine perfusion in rat was previously shown to exert anticonvulsant effects associated with increases in extracellular dopamine and serotonin levels. In contrast, preliminary studies in our laboratory revealed that no anticonvulsant or monoaminergic effects could be obtained after systemic oxcarbazepine administration. The present in vivo microdialysis study was conducted to investigate the impact of the transport kinetics of oxcarbazepine across the blood-brain barrier on the observed treatment refractoriness. More precisely, the influence of intrahippocampal perfusion of verapamil, a P-glycoprotein inhibitor, and probenecid, a multidrug resistance protein inhibitor, on the blood-brain barrier passage and anticonvulsant properties of oxcarbazepine were investigated in the focal pilocarpine model for limbic seizures. Simultaneously, the effects on hippocampal monoamines were studied as pharmacodynamic markers for the anticonvulsant activity. Although systemic oxcarbazepine administration alone failed in preventing the animals from developing seizures, coadministration with verapamil or probenecid offered complete protection. Concomitantly, significant increases in extracellular hippocampal dopamine and serotonin levels were observed within our previously defined anticonvulsant monoamine range. The present data indicate that oxcarbazepine is a substrate for multidrug transporters at the blood-brain barrier. Coadministration with multidrug transporter inhibitors significantly potentiates the anticonvulsant activity of oxcarbazepine and offers opportunities for treatment of pharmacoresistant epilepsy.

Oxcarbazepine (OXC) is the 10,11-keto analog of carbamazepine (CBZ). Like CBZ, it is considered to exert its antiepileptic effects by stabilization of the Na+/H+ channels in a voltage-, frequency-, and time-dependent manner. OXC can also block high-threshold Ca2+ currents and increase K+ channel conductance (McLean et al., 1994). Previously, we showed that increases in hippocampal dopamine (DA) and serotonin (5-HT) levels can have important anticonvulsant effects in the focal pilocarpine model for limbic psychomotor seizures (Clinckers et al., 2004a). These anticonvulsant effects were restricted to a well defined anticonvulsant concentration range and were proven to be mediated by D2 and 5-HT1A receptor stimulation. Anticonvulsant activity against pilocarpine-induced seizures can also be achieved by intrahippocampal perfusion of OXC (unpublished data). From the anticonvulsant threshold concentration (i.e., 100 μM) onward, significant increases in extracellular (EC) hippocampal DA and 5-HT levels were observed, within the previously determined anticonvulsant monoamine ranges. These monoaminergic neurotransmitter increases were again shown to importantly contribute to the anticonvulsant effect via D2.

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ABBREVIATIONS: OXC, oxcarbazepine; CBZ, carbamazepine; DA, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); EC, extracellular; AED, antiepileptic drug; MDT, multidrug transporter(s); BBB, blood-brain barrier; MRP, multidrug resistance protein; Pgp, P-glycoprotein; mCBZ; 2-methyl-5H-dibenz[b,f]azepine-5-carboxamide; SSS, seizure severity score; TSSS, total seizure severity score; LC, liquid chromatography; ANOVA, analysis of variance.
and 5-HT$_{1A}$ receptor stimulation. We therefore defined the hippocampal DA and 5-HT levels as pharmacodynamic markers for the anticonvulsant activity of OXC.

In contrast to intrahippocampal OXC perfusion, preliminary studies have revealed that neither anticonvulsant effects nor hippocampal monoamine increases could be obtained after systemic OXC administration. This discrepancy may be due to the transport kinetics of OXC yielding insufficient OXC concentrations, and as a consequence, insufficient monoamine levels, among other effects, at the seizure focus. On the other hand, since we have shown that hippocampal DA and 5-HT levels are critically involved in the anticonvulsant activity of OXC, the lack of effect after systemic administration might also originate from pharmacodynamic interactions with other brain areas suppressing hippocampal monoamine increases.

Various antiepileptic drugs (AEDs) are proven to be substrates for multidrug transporters (MDT) at the level of the blood-brain barrier (BBB) (Potschka and Löschter, 2001; Potschka et al., 2001, 2002, 2003). These ATP-dependent efflux pumps are highly promiscuous and actively limit brain accumulation of xenobiotics and drugs by transporting these molecules from the basolateral to the apical side of the endothelial cells of the BBB (Kwan et al., 2003). They limit the access of AEDs to the seizure focus. Indeed, several MDT, including P-glycoprotein (Pgp) and members of the multidrug resistance protein family (MRPs), are overexpressed in epileptogenic brain tissue from pharmacoresistant patients. They are considered a possible cause for refractoriness to anticonvulsants (Tishler et al., 1995; Sisodiya et al., 2002). Several other efflux transporters have been found in a variety of tissues, including the brain (Kwan and Brodie, 2005). It is possible that after systemic OXC administration insufficient brain concentrations are obtained due to these active efflux transport mechanisms. However, to our knowledge, it is still unknown whether OXC is a substrate for these MDTs.

The present study was conducted to investigate the impact of the transport kinetics of OXC across the BBB on the observed treatment refractoriness. More precisely, we wanted to investigate the influence of multidrug transporter inhibitors on the BBB passage, anticonvulsant activity, and monoaminergic effects of OXC. Therefore, we used intracerebral microdialysis in a rat model, which has been used to study Pgp- or MRP-mediated transport of AEDs at the BBB (Löschter and Potschka, 2002). Two microdialysis probes were implanted in the left and right hippocampus. The left side was perfused with a Pgp or MRP inhibitor, whereas the right side served as the control side. After systemic administration, this model allows to compare the penetration of OXC into the treated and untreated hemisphere. Simultaneously, this model allows to compare the penetration of OXC into the hippocampus. For the pilocarpine control experiments and the experiments with local Pgp or MRP inhibitor perfusion, a second probe was implanted — 4.6 mm lateral to bregma according to the procedure described above. Probe positioning was histologically verified.

In rats receiving systemic OXC administration, an i.p. catheter for less stressful OXC administration was inserted. The catheter was subcutaneously tunnelled and exited at the neck. The experimental protocol was started at a minimum of 16 h after surgery to allow the animals to recover sufficiently. In this time period, BBB integrity, cerebral blood flow, and glucose metabolism is repaired and normal neurotransmitter levels are measured (Benveniste and Huttemeier, 1990). The freely moving rats were housed in experimental cages with access to food and water ad libitum. During the experiment, the perfusion flow rate was kept constant at 2 $\mu$/min. All microdialysis samples were collected every 20 min, except for the four samples immediately after OXC administration, which were collected every 10 min. Thirty minutes before the start of all experiments (i.e., before the start of basal collections), 1 $\mu$/mL mCBZ was added to the perfusion fluid as an internal standard (in vivo calibrator) to correct for probe recovery variations (Van Belle et al., 1993). mCBZ perfusion did not
affect monoaminergic transmission and did not exert any anticonvulsant activity (data not shown).

All microdialysis protocols started with the perfusion of modified Ringer’s during six collection periods to obtain stable basal hippocampal DA and 5-HT levels (samples 1–6). Afterward, pharmacological manipulations were performed according to the following protocols.

**Group 1: Control Group Experiments.** For the pilocarpine control group experiments (n=6), Ringer’s was perfused through the right hippocampal probe (samples 1–18), and 10 mM pilocarpine, a muscarinic agonist, was perfused through the left probe for 40 min to provoke limbic seizures (samples 12–13). To study the impact of Pgp or MRP inhibitors on pilocarpine-induced seizures, this protocol was repeated with continuous coperfusion of verapamil (5 mM; Kᵢ = 30 μM) (n=4) or probenecid (10 mM; Kᵢ = 44 μM) (n=4) through the left probe (samples 7–18) (i.e., Pgp and MRP control group) (Achira et al., 1999; Sawchuk and Elmqist, 2000). It is assumed that about 10 to 15% of each drug dose diffuses from the microdialysis probes into the surrounding tissue (CMA/Microdialysis).

**Group 2: Intraperitoneal Dose-Response Experiments.** After six basal samples, rats were given an i.p. bolus injection of OXC at the start of sample 7. Thirty minutes later, convulsions were provoked by addition of pilocarpine to the perfusion fluid for 40 min (at the start of sample 10). The following OXC doses were tested: 10, 20, 40, 60, 80, 100, 150, and 200 mg/kg. OXC was suspended in a mixture of propylene glycol, ethanol, and saline (6:2:2). Control injections with this solvent mixture were performed to exclude vehicle effects, but no changes in the EC hippocampal monoamine levels or pilocarpine-induced seizure severity were observed (data not shown).

**Group 3: Influence of Pgp Blockade on Hippocampal OXC and Monoamine Levels (n=6).** The experiment started with an i.p. bolus injection of 100 mg/kg OXC (at the start of sample 7). The right hippocampus was continuously perfused with modified Ringer’s and functioned as control side, whereas the left hippocampal probe was perfused with 5 mM verapamil. Verapamil perfusion was started 30 min before OXC administration and was maintained until the end of the experiment.

**Group 4: Influence of MRP Blockade on Hippocampal OXC and Monoamine Levels (n=6).** The same protocol as described for group 3 experiments was applied but with continuous perfusion of 10 mM probenecid through the left probe.

**Group 5: Influence of Pgp Blockade on the Anticonvulsant Action of OXC (n=6).** Based on previous results, 100 mg/kg OXC was selected as a nonanticonvulsant dose with potential anticonvulsant properties when combined with verapamil perfusion. The protocol described for group 3 was repeated combined with 40-min coperfusion of pilocarpine 30 min after OXC administration.

**Group 6: Influence of MRP Blockade on the Anticonvulsant Action of OXC (n=6).** The protocol described for group 4 was repeated with pilocarpine perfusion. The 10-min dialysate samples were analyzed for OXC and internal standard (mCBZ) content, whereas all the 20-min collections were split for supplementary DA and 5-HT determination.

**Plasma Concentration of OXC**
In group 3 and 4 rats, a catheter was implanted in the femoral artery with the outlet exiting at the neck to facilitate blood sampling. The catheter was continuously flushed with physiological saline (5 μL/min). Whole blood samples (200 μL) were drawn into 1.5-ml polypropylene tubes (VWR International, Leuven, Belgium) containing 20 μL of 5 mM EDTA at 4, 14, 24, 34, 49, 69, 89, 109, 129, 149, 169, and 189 min. The sampling intervals are chosen in correspondence to the time-averaged and lag time-corrected microdialysis sampling times. Blood samples were centrifuged at 12,000 rpm for 3 min, and 100 μl of plasma was finally ultrafiltered (Vivaspin 0.5-ml concentrator, 5000MWCO PES; Vivasience AG, Hannover, Germany) at 5400 rpm for 15 min to obtain free OXC plasma concentrations. Ultrafiltration parameters were optimized, yielding 96% recovery or more.

**Seizure Severity Score**
During each collection period, seizure severity was assessed based on the observation of behavioral manifestations. We adapted the seizure severity score (SSS) from Racine’s scale (Racine, 1972) to take into account the typical behavioral changes associated with pilocarpine-induced motor seizures. This scale consists of six stages that correspond to the successive developmental stages of motor seizures: 0, normal nonepileptic activity; 1, snout and facial movements, hyperactivity, grooming, sniffing, scratching, and wet dog shakes; 2, head nodding, staring, and tremor; 3, forelimb clonus and forelimb extension; 4, rearing and salivating; and 5, falling and status epilepticus. Seizure severity was then determined by summation of the SSSs of each collection period, resulting in a total seizure severity score (TSSS) for each individual animal.

**Chromatographic Assays**
An off-line microbore (100 × 1 mm i.d.) isotropic liquid chromatography (LC) assay coupled to a U-shaped high-sensitivity UV optical cell (cell volume, 70 nl; optical path length, 8 mm) was used to determine OXC and mCBZ in dialysate and ultraltral filtrate samples. The LC-UV system consisted of a Gilson 307 piston pump (Gilson, Villiers le Bel, France). The inlet of the UniJet microbore column (C8, 5 μm; 150 × 1 mm; Unijet, BAS Bioanalytical Systems, West Lafayette, IN) was connected to a Gilson 231 XL sampling injector, and the outlet to a Kontron 433 capillary detector cell (LC Packings, Amsterdam, The Netherlands). The flow rate over the column was 60 μl/min. The mobile phase consisted of filtered (0.2-μm filter) water and acetonitrile (73:27). For both compounds, the limit of quantification was 50 ng/ml. When the samples contained pilocarpine, the limit of quantification was 100 ng/ml.

For the analysis of DA and 5-HT, an off-line microbore LC assay (C8, 5 μm; 100 × 1 mm; Unijet; BAS Bioanalytical Systems) was used with automatic injection (10 μl), as described in detail previously (Sarre et al., 1997).

**Statistical Analysis**
Correct quantitative microdialysis sampling requires the knowledge of the in vivo membrane recovery to obtain accurate estimations of the EC concentrations. Dialysate concentrations for OXC were corrected for in vivo probe recovery via the internal standard technique described by Van Belle et al. (1993). Briefly, at each microdialysis sampling collection the in vivo microdialysis recovery of OXC was estimated by the concomitant loss of mCBZ, the internal standard, from the perfusion fluid. The ratio of the in vivo recovery of OXC to the loss of mCBZ was 0.42 using a 3-mm CMA 12 microdialysis probe. The in vivo probe recovery (mean ± S.E.M.) for OXC was 17.1 ± 1.3% (n=12). The dialysate DA and 5-HT concentrations were corrected for in vivo recovery across the dialysis membrane, estimated by the well established Lonnroth point-of-no-net-flux method (Lonnroth et al., 1987). The microdialysate sampling times were corrected for the microdialysis lag time (i.e., 2 min) and were time-averaged. OXC plasma concentrations were corrected for plasma and ultrafiltration volume variations. The OXC EC hippocampal/plasma concentration ratios were determined both for the left and right hippocampus side and were compared as a measure of the transport across the BBB.

The EC hippocampal and plasma OXC concentrations are expressed as nanomoles per milliliter ± S.E.M. The mean EC DA and 5-HT concentrations are expressed in nanomolar ± S.E.M. Basal transmitter values represent the mean concentrations as obtained under basal conditions, i.e., during the first six collections of each experiment. The acquired TSSSs are represented as the mean TSSS ± S.E.M.

Statistical analysis of the alterations of neurotransmitter and
OXC concentrations and EC hippocampal/plasma ratios between the left and right hippocampus within one group was performed by one-way ANOVA for repeated measurements followed by a Wilcoxon post hoc test for paired replicates ($\alpha = 0.05$). Mann-Whitney $U$ test ($\alpha = 0.05$) was used for comparison of mean neurotransmitter and OXC concentrations and mean TSSSs between groups.

### Results

**Basal DA and 5-HT Concentrations in Hippocampus of Conscious Rats.** Basal EC hippocampal concentrations (mean $\pm$ S.E.M.) corrected for in vivo probe recovery were $0.338 \pm 0.004$ nM for DA ($n = 50$; recovery, $33.31 \pm 3.40\%$) and $0.531 \pm 0.009$ nM for 5-HT ($n = 46$; recovery, $37.62 \pm 3.99\%$).

**Group 1: Impact of Pgp or MRP Blockade on Pilocarpine-Induced Seizure Severity.** Pilocarpine control group results for animals with one hippocampal probe have been described in detail previously (Clinckers et al., 2004a). The presence of a second hippocampal probe did not affect the pilocarpine-induced monoamine profile and seizure severity. Intrahippocampal perfusion of pilocarpine through the left probe evoked full-blown limbic seizures, resulting in a mean TSSS of $14.7 \pm 1.2$ (mean $\pm$ S.E.M.) (Fig. 1). Pilocarpine-induced seizure severity was not affected by verapamil or probenecid coperfusion. The mean TSSS for the verapamil and the probenecid control group was $16.3 \pm 2.4$ and $15.2 \pm 1.4$, respectively (Fig. 1). These values were not significantly different from the mean TSSS for the pilocarpine control group. MRP and Pgp blockade did not influence hippocampal monoamine neurotransmission as evident from a lack of significance between the left and right side EC hippocampal monoamine levels (data not shown).

**Group 2: IP Dose-Response Experiments.** After i.p. administration, the EC hippocampal concentrations of OXC rapidly and dose dependently increased, reaching average maximum levels ($C_{max}$) (Fig. 2) around 50 min ($T_{max}$). None of these systemic OXC doses significantly increased EC hippocampal DA and 5-HT levels or protected the animals from developing seizures (data not shown). The i.p. administration of 150 ($n = 2$) and 200 mg/kg ($n = 2$) OXC was toxic since all animals suffered from respiratory depression and sedation. Therefore, these concentrations were not included in the figures. Systemic administration of 100 mg/kg OXC gave rise to a maximum real EC concentration ($C_{max}$) of $14.07 \pm 1.61$ nmol/ml (Fig. 2). We previously showed that anticonvulsant activity can be obtained from intrahippocampal perfusion with concentrations of $100 \mu$M OXC and higher. Based on the in vivo probe recovery, this corresponds to a real EC hippocampal OXC concentration of approximately 17 nmol/ml (estimation based on the in vivo probe recovery of OXC) (Fig. 2, striped line). This is just above the $C_{max}$ obtained after 100 mg/kg systemic OXC administration. Therefore, 100 mg/kg was selected for further testing with a Pgp or MRP inhibitor.

**Groups 3 and 4: Impact of Pgp or MRP Blockade on Hippocampal OXC and Monoamine Levels.** No statistical differences in control side OXC and monoamine concentrations were observed between the verapamil- ($n = 6$) and probenecid- ($n = 6$)-treated animals. Therefore, all control side results were averaged and represented as the mean of 12 experiments in the figures (Figs. 1 and 3). The OXC plasma levels after 100 mg/kg administration reached a mean peak concentration of $388.1 \pm 36.8$ nmol/ml ($T_{max} = 15$ min). The EC OXC levels of the untreated control hippocampus reached an average maximum level of $14.12 \pm 1.42$ nmol/ml (Fig. 4a). The mean ratio between the OXC concentration in hippocampal EC fluid and plasma was $0.08 \pm 0.01$ at the time of individual maximum concentration in the hippocampus (i.e., 50 min) (Fig. 4b). At the control hippocampus side, no significant changes in EC DA or 5-HT levels were observed compared with baseline levels (i.e., concentration at time 0) (Fig. 3a). Local perfusion with verapamil or probenecid in the ipsilateral hippocampus significantly increased the EC OXC levels. The animals did not show respiratory depression and sedation. At the time of individual maximal EC hippocampal OXC levels, a significant difference in increased OXC concen-

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**Fig. 1.** Effect of 100 mg/kg OXC administration with and without verapamil or probenecid cotreatment on pilocarpine-induced seizure severity. Each column represents the mean TSSS for each control and experimental group (mean $\pm$ S.E.M.). An i.p. injection of 100 mg/kg OXC alone did not protect the animals from seizures, as evident from a lack in significant difference in TSSS between the pilocarpine control group and 100 mg/kg group. Administration of 100 mg/kg OXC with intrahippocampal perfusion of verapamil or probenecid resulted in a significant decrease in seizure severity compared with the Pgp and MRP control group. The TSSS values significantly different from the respective control group value are denoted with an asterisk and a dagger, respectively ($p < 0.05$) (Mann-Whitney $U$ test).

**Fig. 2.** Effect of increasing systemic OXC doses (in milligrams per kilogram) on the EC hippocampal OXC levels (in nanomoles per milliliter). For each administered dose, the $C_{max}$ (mean $\pm$ S.E.M.) obtained at the level of the hippocampus is depicted. The striped line depicts the mean EC hippocampal level obtained after 100 $\mu$M OXC intrahippocampal perfusion, i.e., the anticonvulsant threshold concentration for local perfusion in hippocampus.
trations between the verapamil- and probenecid-treated group was obtained. The increase was 39.3 ± 4.9 and 73.8 ± 6.6%, respectively, compared with the control hippocampus side (Fig. 4a). As a consequence, the EC hippocampal/plasma ratio was significantly increased both at the verapamil- and probenecid-treated side. The mean EC hippocampal/plasma ratio was 0.11 ± 0.02 for the verapamil-treated side and 0.14 ± 0.02 for the probenecid-treated side at the time point of individual maximal EC hippocampal concentration. The EC hippocampal monoamine levels at the MDT blocker-treated side also increased. At the verapamil-treated side, DA levels increased 169.9 ± 10.2% and 5-HT levels 157.6 ± 12.9% (Fig. 3b). At the probenecid-treated side, DA and 5-HT levels increased 216.3 ± 20.4 and 203.6 ± 17.8%, respectively (Fig. 3c). In correspondence to the hippocampal OXC levels, significantly different hippocampal monoamine levels were observed between the verapamil- and probenecid-treated animals.

Groups 5 and 6: Impact of Pgp or MRP Blockade on the Anticonvulsant Action of OXC. When i.p. administration of 100 mg/kg OXC was combined with Pgp or MRP blocker perfusion at the site of seizure onset (i.e., hippocampus), the animals were completely protected against seizures. After pilocarpine perfusion, these rats only rarely showed hyperactivity and wet dog shakes, resulting in a mean TSSS of 1.9 ± 0.4 and 2.3 ± 0.6 for the verapamil- and probenecid-treated animals, respectively (Fig. 1).

Discussion

The present data clearly demonstrate that administration of 100 mg/kg OXC with concomitant inhibition of either Pgp
or MRP results in a significant increase in the EC hippocampal OXC concentrations compared with rats receiving only 100 mg/kg OXC. This increase was more pronounced in animals receiving probenecid than in those receiving verapamil ($p < 0.05$). The difference in increased OXC levels between both experimental settings are shown not to be secondary to changes in peripheral drug kinetics since the EC hippocampal/plasma ratios between the different experimental groups increased to a similar extent. Potschka et al. (2001) applied the same inhibitors in the same concentrations to study the BBB passage of carbamazepine and did not observe this difference. These data therefore suggest that OXC has a higher affinity for MRP than Pgp.

The most important finding of the current study is that coadministration of a nonanticonvulsant dose of OXC with local perfusion of either verapamil or probenecid is able to prevent the development of pilocarpine-induced limbic motor seizures. Indeed, the TSSSs for the animals receiving co-treatment with verapamil or probenecid were significantly lower than for those animals receiving only OXC. It is important to note that neither probenecid nor verapamil perfusions affected pilocarpine-induced seizure severity. This, however, could have been expected for verapamil, since voltage-operated Ca$^{2+}$ channels have been suggested as a pharmacotherapeutic drug target to correct the aberrant pathophysiology of epileptogenesis via a phenomenon known as “intrinsinc burst firing” driven by an inward Ca$^{2+}$ current (Kulak et al., 2004). Several Ca$^{2+}$ channel antagonists were shown to possess anticonvulsant potential in experimental and clinical studies (De Sarro et al., 1988; Czuczwar et al., 1990; Larkin et al., 1992; Speckmann et al., 1993; Gasior et al., 1995, 1996; Swieder et al., 2002). However, systemic isradipine and verapamil administration per se or in combination with AEDs failed to display any anticonvulsant activity (Czuczwar et al., 1990; Borowicz et al., 1997). Moreover, verapamil was shown to lack anticonvulsant effects after intracerebroventricular administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures (De Sarro et al., 1988).

The present data also indicate that under physiological conditions, MDTs will actively limit the penetration and accumulation of OXC into the brain. To our knowledge, this has never been shown. We suggest that the observed resistance to OXC treatment in the current rodent seizure model results from insufficient OXC levels reaching the seizure focus. Indeed, in physiological conditions the EC hippocampal/plasma ratio for OXC is approximately 10%. In contrast, for levetiracetam, which was shown not to be a substrate for the currently investigated MDTs, this ratio was about 20% (Potschka et al., 2004). Our data are in line with the EC hippocampal/plasma ratios for other AEDs, which are substrates for MDTs. Increasing the systemic dose to elevate the OXC levels in the brain is not an option, as higher OXC doses are shown to be toxic. By promoting the passage through the BBB via concomitant local perfusion with an MDT inhibitor, anticonvulsant activity was established, whereas no behavioral toxicity was observed. Araujo et al. (2004) investigated the neurotoxic effects of OXC in primary rat hippocampal neurons. After 24-h treatment with 300 μM OXC, they reported degeneration and swelling of neurites and increases in reactive oxygen species. In the current study, the hippocampal OXC concentrations measured via in vivo quantitative microdialysis after 100 mg/kg, with and without MDT inhibitor perfusion, were approximately 14 μM.

The novelty of the current work lies in the concomitant evaluation of the effects of MDT blockade on BBB passage of OXC and its pharmacodynamic effects. Elevation of the EC hippocampal OXC levels was accompanied by significant increases in EC hippocampal DA and 5-HT levels. In accordance with the difference in hippocampal OXC levels between the verapamil and probenecid experimental groups, these concomitant monoamine increases were more pronounced in the latter group. The EC DA and 5-HT levels were situated within the previously determined anticonvulsant monoamine range and were shown to contribute, at least partly, to the anticonvulsant effect of OXC (unpublished data). Intrahippocampal perfusion with the anticonvulsant threshold concentration of OXC was associated with significant increases in EC hippocampal monoamine levels within the protective range (unpublished data). These data are in accordance with previous data in which we observed the same association for exogenously perfused monoamines and selective monoamine reuptake blockers (Clinckers et al., 2004a,b). The current data indicate that after systemic OXC administration, anticonvulsant activity is still accompanied by significant increases in EC hippocampal monoamine levels. Moreover, these effects on hippocampal monoamine release indicate that the observed resistance to OXC treatment does not originate from a pharmacodynamic interaction with other brain areas suppressing monoaminergic release at the seizure focus, as hypothesized in the Introduction.

Besides OXC, several other antiepileptic compounds are also transported by both MDT families (Löschner and Potschka, 2002), suggesting that Pgp and MRPs have overlapping substrate spectra. This is already well described in substrate recognition studies (Lee et al., 2001). In the current study, “first generation” modulators were applied, which lack transporter specificity. In addition to Pgp, verapamil interacts with organic anion transporter 2 and organic cation/carnitine transporter 1, whereas probenecid is an inhibitor of MRP 1, 2, and 5; organic anion transporter 1 and 3; organic anion-transporting polypeptide 1 and 2; and monocarboxylic acid transporter 1 ( Sugiyama et al., 2001; Gerk and Vore, 2002; Taylor, 2002). In contrast, some investigators have suggested that probenecid stimulates MRP2 activity ( Bakos et al., 2000). Moreover, interactions with other MDTs at the BBB, which have not yet been identified, may be involved. Therefore, this lack of transporter specificity may be responsible for the currently reported substrate overlap. Indeed, the observed increases in EC OXC brain levels in response to verapamil and probenecid perfusion may well originate from an inhibitory effect on mutual transporters. However, the use of verapamil and probenecid in the current experimental setting does not always result in overlapping substrate specificity. Potschka et al. (2002, 2003, 2004) applied the same model to investigate the involvement of Pgp and MRPs in the penetration of phenobarbital and levetiracetam across the BBB. Only Pgp but not MRP inhibition was shown to increase the EC phenobarbital brain levels, whereas EC levetiracetam levels were not affected by any of the MDT inhibitors. Due to the lack of specificity of the applied inhibitors it is impossible to define the exact MDT family or subtype that is involved. For example, Potschka et al. (2003) showed that coadministration of probenecid resulted in an increase in
brain access of CBZ. However, they could not detect an impact of MRPs2 deficiency on CBZ brain penetration in MRPs2-deficient TR− rats. It was concluded that the effects of probenecid on the brain penetration of CBZ were related to the inhibition of MDTS other than MRPs2. Nevertheless, we are convinced that the current protocol at least allows us to conclude that MDTs are involved in the BBB passage of OXC and that transporter inhibition is able to potentiate OXC effects. However, we recognize that in the future more specific transport inhibitors should be used to unravel which specific efflux transporter subtypes are involved.

In conclusion, we applied quantitative in vivo microdialysis to study the transport kinetics of OXC across the BBB. Simultaneously, the real EC drug concentrations were related to their responses on EC monoamine concentrations. We demonstrated that MDTs are importantly involved in the regulation of brain EC levels of OXC and its pharmacodynamic response. Transporter inhibition via verapamil and probenecid administration is shown to potentiate the anti-convulsant effect of OXC. Our data are in line with previous results for other AEDs and suggest the addition of a transporter inhibitor to current therapy with AEDs as an option for the treatment of refractory epilepsy. Systemic administration of 50 mg/kg probenecid is already shown to potentiate the effects of drug to the brain in rodents (Potschka et al., 2003). Moreover, Summers et al. (2004) recently reported greatly improved overall seizure control in a pharmacoresistant patient after addition of verapamil to the AEDs regimen, despite the known poor penetration of verapamil through the BBB (Hamann et al., 1983). However, as reported previously not all AEDs are substrates for both MDTs. To improve the success rate of cotreatment with efflux transporter inhibitors in refractory epilepsy patients, more studies should be conducted to determine the substrate spectra of different MDT subtypes by the use of newer generation, more specific transport inhibitors. In the future, we will investigate the effect of systemic coadministration with newer generation MDT blockers on the BBB passage and anti-convulsant activity of OXC and other AEDs.

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