In Vivo Blood-Brain Barrier Transport of Oxycodone in the Rat: Indications for Active Influx and Implications for Pharmacokinetics/Pharmacodynamics

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ABSTRACT:

The blood-brain barrier (BBB) transport of oxycodone was studied in rats. Microdialysis probes were inserted into the striatum and vena jugularis. Ten animals were given a bolus dose followed by a 120-min constant rate infusion to study the steady-state concepts of oxycodone BBB equilibration. Another 10 animals were given a 60-min constant rate infusion to study the rate of equilibration across the BBB. Oxycodone-D3 was used as a calibrator for the microdialysis experiments. The samples were analyzed with a liquid chromatography-tandem mass spectrometry method and a population pharmacokinetic model was used to simultaneously fit all the data using NONMEM. A two-compartment model which allowed for a delay between the venous and arterial compartments best described the pharmacokinetics for oxycodone in blood and plasma, whereas a one-compartment model was sufficient to describe the pharmacokinetics in the brain. The BBB transport of oxycodone was parameterized as CLin and $K_{p,uu}$. CLin describes the clearance of oxycodone across the BBB into the brain, whereas $K_{p,uu}$ describes the extent of drug equilibration across the BBB. CLin across the BBB was estimated to 1910 $\mu$L/min · g brain. $K_{p,uu}$ was estimated to 3.0, meaning that the unbound concentration of oxycodone in brain was 3 times higher than in blood, which is an indication of active influx of oxycodone at the BBB. This is the first evidence of an opioid having an unbound steady-state concentration in brain that is higher than unity, which can explain potency discrepancies between oxycodone and other opioids.

The blood-brain barrier (BBB) is composed of capillary endothelial cells connected by tight junctions. Its main function is to be a physical and active barrier to restrict and regulate the penetration of compounds into and out from the brain to maintain brain homeostasis. Transport into the brain across the BBB is essential for drugs that act within the central nervous system (CNS), whereas BBB penetration needs to be minimized for drugs with potential CNS side effects.

Brain distribution can be described with respect to the rate and extent of equilibration of a drug molecule across the BBB (Hammarlund-Udenaes, 2000). The rate of equilibration can be expressed as clearances into and out of the brain, CLin and CLout, respectively. The extent of equilibration across the BBB can be expressed as the ratio of the steady-state concentration of unbound drug in brain over unbound drug in blood, $K_{p,uu}$ (Gupta et al., 2006). $K_{p,uu}$ is equivalent to the ratio of the area under the concentration versus time curve of unbound drug in brain and blood, AUCbrain/AUCblood, as well as to the ratio CLin/CLout. This assumes that it is only unbound drug that crosses the BBB and that metabolism and interstitial bulk flow contribution to brain efflux is minor. The extent of equilibration equals the net flux of drug across the BBB.

Conclusions on the bidirectional transport properties of the BBB can be drawn based on the unbound concentrations in brain and blood (Hammarlund-Udenaes et al., 1997). If $K_{p,uu}$ is unity, the transport across the BBB is predominantly passive diffusion, or the impact of influx and efflux transport is equal. If $K_{p,uu}$ is below unity, a likely explanation is active efflux transport, metabolism at the BBB, or substantial influence of bulk flow on drug brain elimination. A $K_{p,uu}$ above unity is an indication that active influx processes, such as influx transporters, are involved in the BBB transport. Using microdialysis, full time-profiles of unbound drug in both brain and blood can be obtained from each individual. Thus, microdialysis gives direct quantitative information about the in vivo net flux of possible influx and efflux mechanisms.

Oxycodone is an opioid receptor agonist (Monory et al., 1999) used to treat moderate to severe pain. The potency for oxycodone when used in postoperative pain treatment is similar to that of morphine (Silvasti et al., 1998), despite lower affinity to the opioid receptors (Peckham and Traynor, 2006). Given that the affinity to the opioid receptors is lower for oxycodone than for morphine, a higher concentration of oxycodone would be necessary to give rise to a similar effect. By observing only plasma concentrations, it is therefore unclear how the two opioids can be equipotent after systemic administration. Oxycodone has been used clinically for the past 90 years, but little is known about its BBB transport properties. We recently showed that coadministration of the P-glycoprotein inhibitor valsepo-

ABBREVIATIONS: BBB, blood-brain barrier; NONMEM, nonlinear mixed effects model; CNS, central nervous system; RBC, red blood cell; RSE, relative standard error; ISF, interstitial fluid; CL, clearance; AUC, area under the concentration curve.
Materials and Methods

Animals. Male Sprague-Dawley rats (B&K, Sollentuna, Sweden) weighing 250 to 290 g were used. The animals were group-housed at 22°C under a 12-h light/dark cycle for at least 5 days before the experiment. Food and water were available ad libitum. The study was approved by the Animal Ethics Committee of Tierp District Court, Tierp, Sweden (C 247/1).

Chemicals. Oxycodone hydrochloride and phenobarbital were obtained from Apoteket AB, Production and Laboratory (Umeå, Sweden). Oxycodone-D3 and -D6, noroxycodone, noroxycodone-D3, oxymorphone, and oxymorphone-D3 were purchased from Cerilliant Corporation (Round Rock, TX). All chemicals were of analytical grade. All solvents were of high-performance liquid chromatography grade.

In Vitro Blood/Plasma Partition of Oxycodone. The partition of oxycodone between blood and plasma was examined in vitro. The experimental setting has been described previously (Kerbusch et al., 2001). Fresh rat blood spiked with two oxycodone concentrations (50 ng/ml and 500 ng/ml) were placed in a thermostatically controlled water bath at 37°C. At various time points (0, 15, and 60 min), samples were taken and centrifuged for 2 min at 10,000 rpm. After centrifugation, the plasma layer was separated from the red blood cell (RBC) layer and the RBCs were transferred to clean vials. All samples were stored at −20°C pending analysis. The experiment was performed in duplicates of each concentration.

Animal Surgery. The animals were anesthetized by inhalation of enflurane (Efrane; Abbott Scandinavia AB, Kista, Sweden). A PE-50 cannula fused with Silastic tubing was inserted into the left femoral vein for oxycodone administration. A PE-50 cannula fused with a PE-10 tubing was inserted into the femoral artery for blood sampling. To avoid clotting, these catheters were filled with a heparinized saline solution (Heparin Leo, 100 IU/ml; Leo Pharma AB, Malmö, Sweden). A CMA/20 blood probe (10 mm; CMA, Stockholm, Sweden) was inserted into the right jugular vein through a guide cannula and fixed to the pectoralis muscle with two sutures. The anesthetized rat was placed into a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) for the implantation of the brain probe. A mid sagittal incision was made to expose the skull, and the CMA/12 guide cannula was implanted into the striatum with the coordinates 2.7 mm lateral and 0.8 mm anterior to the bregma and 3.8 mm ventral to the surface of the brain. After insertion, the guide cannula was anchored to the skull with a screw and dental cement (Dentalon Plus; Heraeus, Hanau, Germany). A CMA/12 probe was inserted into the striatal guide. A 15-cm piece of PE-50 tubing was looped subcutaneously on the back of the rat to the surface of the neck to let the perfusion solution adjust to body temperature before entering the brain probe. All ends of the cannulae and catheters were passed subcutaneously to a plastic cup placed on the posterior surface of the neck out of reach from the rat. During the surgical procedure, the rat body temperature was maintained at 38°C by using a CMA/150 temperature controller. The rats were placed in a CMA/120 system for freely moving animals with free access to water and food, and were allowed to recover for approximately 24 h. All experiments were performed at the same time of the day.

Bolus Dose Plus Constant Rate Infusion Regimen. Ten animals were given a bolus dose followed by a 120-min constant rate infusion. The blood and brain microdialysis probes were perfused with Ringer solution (147 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, and 0.85 mM MgCl2) containing 45 ng/ml of the calibrator oxycodone-D3 at a flow rate of 1 μl/min by using a CMA/100 precision infusion pump. Samples were collected in 15-min intervals. After a 60-min stabilization period, oxycodone was administered as an intravenous bolus dose of 0.277 mg/kg over 10 s, followed by a 120-min constant infusion of 0.533 mg/kg/h in the left femoral vein by using a Harvard 22 pump (Harvard Apparatus Inc., Holliston, MA). The animals were studied two at a time and the dose was calculated based on the average weight of the two animals. Brain and blood dialysates were collected in 10-min intervals during the first hour of the infusion and in 20-min intervals during the last hour of the infusion. Five rats were given an overdose of phenobarbital right before the end of the infusion and were decapitated for determination of total brain oxycodone concentrations at steady state. After the end of the infusion, brain and blood dialysates for the remaining five rats were collected in 10-min intervals during 1 h and in 20-min intervals during the last hour of the experiment.

Constant Rate Infusion Regimen. Another 10 animals were given a constant rate infusion of oxycodone. Both microdialysis probes were perfused with Ringer solution (147 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, and 0.85 mM MgCl2) containing 45 ng/ml of the calibrator oxycodone-D3 at a flow rate of 1 μl/min by using a CMA/100 precision infusion pump. Samples were collected in 15-min intervals. After a 60-min stabilization period, oxycodone was administered as an intravenous constant rate infusion of 0.3 mg/kg over 60 min in the left femoral vein by using a Harvard 22 pump (Harvard Apparatus Inc.). Brain and blood dialysates were collected in 10-min intervals during the infusion and for the first hour after the stop of the infusion. Thereafter, the dialysates were collected in 20-min intervals for the last 2 h of the experiment.

One to eight blood samples from each rat were collected into heparinized Eppendorf vials (Brand, Wertheim, Germany) at predose and 5, 10, 20, 30, 60, 75, 90, 105, 120, 130, 140, 180, or 240 min after the start of the oxycodone infusion. No more than 2 ml of blood was collected from each rat.

After the end of the collection interval, microdialysates were capped and stored at −20°C until analysis. The blood samples were centrifuged at 10,000 rpm for 7 min, and the plasma was transferred to Eppendorf vials (Brand). All brains were examined to ensure that there was no extensive bleeding around the brain probe. Plasma samples and brains were stored at −20°C until analysis.

Sample Preparation. The Ringer samples were diluted with an equal volume of water spiked with the internal standard oxycodone-D6 at a concentration of 40 ng/ml, vortexed for 5 s, and 16 μl was injected onto the column. Fifty microliters of plasma was precipitated with 100 μl of acetonitrile spiked with the internal standard at a concentration of 30 ng/ml and vortexed for 5 s. After centrifugation at 10,000 rpm for 5 min, 30 μl of the supernatant was injected onto the column.

Each individual brain tissue sample was pretreated as described previously (Bodström et al., 2004). The sample was homogenized with a 5-fold volume of 0.1 M perchloric acid. The homogenates were centrifuged for 10 min at 3000 rpm. One hundred microliters of the supernatant was pretreated using a slightly modified solid phase extraction method by Joel et al. (1988). Solid phase extraction columns were coupled to a vacuum manifold and conditioned. One hundred microliters of brain homogenate supernatant was vortexed with 20 μl of water containing the internal standard and 3 ml of 0.5 M ammonium sulfate (pH 9.5) for 5 s. Thereafter, the samples were transferred to the column and drawn through the cartridge. The column was washed with 20 ml of 5 mM ammonium sulfate (pH 9.5), 0.5 ml of water, and 100 μl of methanol, before the analytes were eluted three times with 2 ml of methanol. A stream of nitrogen and a temperature of 45°C were used to evaporate the eluate, and the residue was dissolved in 100 μl of the mobile phase. Forty microliters of the reconstituted sample was transferred to a polyethylene autosampler vial and 30 μl was injected onto the column.

The RBCs were prepared according to a previously described method (Dumez et al., 2005). The RBCs were diluted with 4 times its volume with distilled water. After 5 min, the solution was vortexed and thereafter centrifuged at 10,000g to precipitate the cell debris. Four hundred microliters of the solution was conducted to the same solid phase extraction procedure as for the brain homogenate supernatant.

Drug Quantification. Oxycodone and the microdialysis probe calibrator oxycodone-D3 were quantified using a liquid chromatography-tandem mass spectrometry method (Boström et al., 2004). Oxycodone-D6 was used as internal standard. The liquid chromatography-tandem mass spectrometry system consisted of an LC-10AD pump (Shimadzu, Kyoto, Japan) and a Triathlon 900 autosampler (Spark Holland, Emmen, The Netherlands) equipped with a 100-μl loop and a Zorbax SB-CN column (4.6 × 150 mm; Agilent Technologies, Wilmington, DE). The mobile phase consisted of 45% acetonitrile in 5 mM ammonium acetate. The column was maintained at 50°C and a constant flow rate of 1.0 ml/min was used. The flow was split, allowing 0.2 ml/min to enter the mass spectrometer (Quattro Ultima; Micromass, Manchester, UK). The transition modes for oxycodone, oxycodone-D3, and oxycodone-D6 were set to m/z 316.1 to m/z 297.9, m/z 319.1 to m/z 301, and m/z 322.2 to m/z 304, respectively. Mass spectrometry control and spectral processing were carried out using MassLynx software, version 4.0 (Micromass).
All standard curves of oxycodone and oxycodone-D3 in different matrices showed good linearity (coefficient of determination >0.990). The limit of quantification of oxycodone and oxycodone-D3 was 0.5 ng/ml in Ringer and plasma. In RBCs and total brain, the limit of quantification was 5 ng/ml and 20 ng/ml, respectively. Runs were accepted if the precision and accuracy of the quality control samples had a coefficient of variation (CV) below 15%.

Data Analysis. To estimate the CL,in of oxycodone across the BBB, unbound brain and blood concentrations as well as total plasma concentrations were modeled using population nonlinear mixed effects modeling by NONMEM (version VII) (GloboMax LLC, Hanover, MD). The method resolves both inter- and intra-animal variability in the parameters as well as the residual variability between observations and the model predictions. The first-order conditional estimation with interaction method was used throughout the modeling procedure. The subroutine ADVAN6 was used for the NONMEM analysis. The model selection was based on the objective function value, pharmacokinetic parameter point estimates, standard errors, and scientific plausibility, as well as graphical analysis using Xpose 3.104 (Jonsson and Karlsson, 1999) implemented into S-plus 6.1 (Insightful Corp., Seattle, WA). To distinguish between two nested models, a drop in the objective function value of 6.63 was required. This value (χ² distributed) corresponds approximately to p < 0.01 for a one-parameter difference. An exponential variance model was used to describe the interanimal variability:

\[ P_i = P_{pop} \cdot \exp(\eta) \]  

where \( P_i \) and \( P_{pop} \) are the parameter in the \( i \)th animal and the typical animal, respectively. \( \eta \) is the interanimal variability, assumed to be normally distributed around zero and with a standard deviation, \( \omega \), to distinguish the \( i \)th animal’s parameter from the typical value as predicted from the regression model. The need for interanimal variability was investigated in all model parameters.

The percentage relative standard error (RSE%) of the fixed effects parameter estimates was expressed as the standard error (S.E.) divided by the parameter estimate times 100. The RSE (%) of the random effects parameter estimates were expressed as the S.E. divided by the parameter estimate on the variance scale. Additive, proportional, and slope-intercept models were considered for the residual variability. Different error models were explored for each of the five observation types. The possibility of joint residual errors for the different observation types was also investigated. The residual errors were assumed to be normally distributed around 0.

Initially, submodels were developed separately to speed up model development and facilitate location of model misspecification. The model developed was based on the integrated blood-brain pharmacokinetic model for morphine with some modifications (Tunblad et al., 2004). The blood probe recovery and blood dialysate data were combined and a submodel was developed. Thereafter, the total plasma concentration data were included and, subsequently, also the brain probe recovery data and the brain dialysate concentrations. Each part of the model was reevaluated after adding each observation type. Finally, all the data were simultaneously fitted. The final model is depicted in Fig. 1. One- and multicompartmental pharmacokinetic models were considered for both systemic and brain pharmacokinetics. To allow for a distribution delay between the arterial and venous compartments, the central unbound volume of distribution (\( V_u \)) was equally divided between central arterial and venous volumes, V A and V V. Elimination was assumed to occur only from the arterial compartment. The equations describing the model can be found in Tunblad et al. (2004), with the change that the BBB transport was parameterized in terms of \( CL_{in} \) and \( K_p,u,u \) was equivalent to \( CL_{in}/CL_{out} \) (Hammarlund-Udenaes et al., 1997; Gupta et al., 2006). \( CL_{in} \) and \( K_{p,u,u} \) were estimated as:

\[ CL_{in} = k_{out} \cdot VA \]  

\[ CL_{out} = k_{out} \cdot V_u,brain \]  

\[ K_{p,u,u} = \frac{CL_{in}}{CL_{out}} \]  

where \( V_u,brain \) is the unbound volume of distribution in the brain.

This individual unbound volume of distribution in the brain was calculated using:

\[ V_u,brain = (A_{brain} - V_{blood} \cdot C_{blood})/C_{brain} \]  

where \( A_{brain} \) is the total amount of oxycodone per gram of brain at steady state. \( V_{blood} \) is the volume of blood per gram of brain. \( C_{brain} \) is the unbound brain interstitial fluid (ISF) concentration at steady state. \( A_{blood} \), \( C_{blood} \), and \( C_{brain} \) were calculated using data obtained from rats decapitated at the end of the infusion (n = 5) in the bolus + constant rate infusion regimen. \( C_{blood} \) is the total concentration in blood that can be assessed by multiplying the plasma concentration with the partitioning between plasma and blood (\( C_{blood}/C_{plasma} \)), which was calculated according to Eq. 6 (Tozer, 1981):

\[ C_{blood} = \frac{C_{plasma}}{1 - H + H \cdot (C_{RBC}/C_{plasma})} \]  

where \( C_{blood} \), \( C_{plasma} \), and \( C_{RBC} \) are the oxycodone concentration in blood, plasma, and red blood cells, respectively. \( H \) is the hematocrit, estimated to 42% in the rat (Leonard and Ruben, 1986). The volume of blood in the rat brain was fixed to 14 μl/g brain (Bickel et al., 1996). The average value of \( V_u,brain \) from five animals was used as a fixed parameter in the model. \( V_u,brain \) and \( C_{blood}/C_{plasma} \) are presented as averages with S.D.

The final model was validated by running a bootstrap validation procedure. Two hundred datasets were generated, randomly sampled from the original dataset with replacement. Then each of the datasets were fitted to the final pharmacokinetic model, and the mean, S.E., and 95% confidence intervals of the model parameters were calculated and compared with the final parameter estimates and S.E. generated by NONMEM.

To be able to make comparisons with blood flow, blood clearance across the BBB (\( CL_{in,blood} \)) was calculated using eq. 7:

\[ CL_{in,blood} = \frac{CL_{in} \cdot f_d}{C_{plasma}} \]  

where \( CL_{in} \) and \( f_d \) are the final model estimates of the fixed effects and \( C_{blood}/C_{plasma} \) are calculated from eq. 6. \( CL_{out} \) was calculated according to eq. 8:

\[ CL_{out} = \frac{CL_{in}}{K_{p,u,u}} \]  

where \( CL_{in} \) and \( K_{p,u,u} \) are the final model estimates of the fixed effects.

The partitioning of drug between brain and blood at steady state can be calculated as:

\[ K_p = \frac{C_{brain}}{C_{plasma}} \]  

\[ K_{p,u} = \frac{C_{brain}}{C_u} \]  

\[ K_{p,u,u} = \frac{C_{brain,ISF}}{C_u} \]  

where \( K_p \) is the partition coefficient between total brain concentrations (\( C_{brain} \)) and total plasma concentrations (\( C_{plasma} \)). \( K_{p,u} \) is the partition coefficient between total brain concentrations (\( C_{brain} \)) and unbound blood concentrations (\( C_u \)). \( K_{p,u,u} \) is the partition coefficient between unbound drug in ISF and unbound drug in blood. \( K_p, K_{p,u} \), and \( K_{p,u,u} \) are presented as averages with S.D.

Results

After the bolus dose plus constant rate infusion regimen, oxycodone steady-state concentrations in plasma and blood dialysate were reached rapidly. The brain dialysate concentrations also rapidly reached steady state, where the unbound oxycodone concentrations in brain ISF were higher than those in blood (Fig. 2A). To achieve stable estimates of \( CL_{in} \), there was a need for information on the time aspects of oxycodone equilibration across the BBB. Therefore, a constant rate infusion was given. Higher unbound brain concentrations compared
with blood were also seen after the constant rate infusion regimen (Fig. 2B).

\[ K_{\text{puu}} = 3.03 (0.038). \]

After the initially even higher brain ISF concentrations, the ratio decreased and remained at 3 throughout the experiment, i.e., over a wide range of concentrations (Fig. 3). No difference was found between the two dosage regimens during or after the end of the infusion. After the end of the infusions, the unbound concentrations in brain and blood declined with the same half-life. The partition coefficients \( K_p \) and \( K_{pu} \) were 4.5/1.1006 and 7.8/1.2, respectively. The total brain, plasma, and unbound blood oxycodone concentration at steady state was 484/169 ng/g brain, 84/16 ng/ml, and 62/9 ng/ml, respectively. This resulted in an average \( V_u,\text{brain} \) of 2.20 ± 0.53 ml/g brain.

The partitioning of oxycodone between blood and plasma resulted in a \( C_{\text{blood}}/C_{\text{plasma}} \) ratio of 1.3 ± 0.3. The binding was not concentration-dependent in the range 50 to 500 ng/ml. Equilibrium was achieved instantaneously and was stable during the study period of 60 min.

A two-compartment model that allowed for a delay between the venous and arterial compartments best described the pharmacokinetics for oxycodone in blood and plasma, whereas a one-compartment model was sufficient to describe the pharmacokinetics in the brain (Fig. 1). The influx clearance across the BBB, \( CL_{in} \), was estimated to 1910 (0.20) \( \mu l/min \cdot g \) brain. Using eq. 7, this corresponds to a \( CL_{in,\text{blood}} \) of 1092 \( \mu l/min \cdot g \) brain. \( CL_{out} \) was calculated to 630 \( \mu l/min \cdot g \) brain. The final model parameters are given in Table 1 and were all estimated with high precision. The data supported interanimal variability in blood and brain recovery, \( CL, V_1, \) and \( f_u. \)

A slope intercept model with variances \( \sigma^2_{\text{prop,brain}} \) and \( \sigma^2_{\text{add,brain}} \) was used to describe the brain dialysate data. Proportional error models with variances \( \sigma^2_{\text{prop,blood}} \) and \( \sigma^2_{\text{prop,plasma}} \) were used to describe the residual variability in the blood dialysate and plasma data, respectively. Additive error models with variances of \( \sigma^2_{\text{add,REC,blood}} \) and \( \sigma^2_{\text{add,REC,brain}} \) were used to describe the residual variability in the blood and brain recovery data, respectively.

The final parameter estimates for the bootstrap validation were in good agreement with the estimates of the final model (Table 1). The goodness of fit plots for the different observation types are shown in Fig. 4. Good, medium, and poor fits for the blood dialysate concentrations, brain dialysate concentrations, and plasma concentrations are shown in Fig. 5.

**Discussion**

In this study, oxycodone showed a \( K_{\text{puu}} \) of 3, suggesting that the influx clearance is 3-fold greater than the efflux clearance. This is the
first evidence of an opioid having an unbound steady-state concentration in brain that is higher than in blood. The finding indicates that oxycodone is actively influxed at the BBB. Other opioids have much lower ratios: morphine 0.29 (Tunblad et al., 2003), morphine-6-glucuronide 0.27 (Tunblad et al., 2005), morphine-3-glucuronide 0.10 (Xie et al., 2000), and codeine 1.0 (Xie and Hammarlund-Udenaes, 1998). The much higher ratio of oxycodone is in spite of the similar lipophilic properties of oxycodone and morphine (Peckham and Traynor, 2006). The very lipophilic drug diazepam, which is generally thought to pass membranes only by passive diffusion, showed an unbound concentration ratio between brain and blood of 1 (Dubey et al., 1989).

Reports comparing the pain-relieving effects of morphine and oxycodone conclude that the two opioids are equipotent (Silvasti et al., 1998). This is in contrast to the lower affinity for the /H11001-H9262-opioid receptor for oxycodone than for morphine (Peckham and Traynor, 2006). Because the opioids are active in the CNS, the equipotency of the two drugs may be explained by the oxycodone BBB net flux of 3, as compared with the morphine BBB net flux of 0.29. This results in a higher brain concentration for oxycodone than could be anticipated from plasma concentrations. The knowledge of the net flux across the BBB may therefore be of importance when interpreting pharmacokinetic/pharmacodynamic relationships for CNS drugs, especially when comparing different routes of administration.

It is difficult to speculate about the reason for the rapid and very high brain uptake of oxycodone. Mechanistic information about the transporter responsible for the extensive uptake of oxycodone into the brain would give strong incentives to drug development, since it could provide knowledge about strategies of transporting drugs into the brain. In the present study we have quantified the transport. Other experiments are needed to understand more about the mechanisms with which oxycodone is transported. The initially even higher ratio might suggest that the transport mechanism is saturable. If this were true, one would expect the ratio to increase after the end of infusion. This was not the case for oxycodone.

There are a variety of capacity-limited ATP-dependent transport proteins at the BBB. These include the well known P-glycoprotein and multidrug resistance proteins, which actively efflux drugs at the BBB.
Several CNS drugs such as morphine and methadone are substrates for such efflux transporters at the BBB (Loscher and Potschka, 2005). There is also evidence of transport proteins that are involved in the influx of nutrients to the brain and evidence that drugs such as L-dopa and morphine-6-glucuronide use influx transporters to enter the brain.
There are reports describing peptides (Thomas et al., 1997), gabapentin (Luer et al., 1999), fentanyl (Henthorn et al., 1999), and pentazocine (Suzuki et al., 2002) using saturable mechanisms to enter the brain. However, a net steady-state flux between brain and blood above unity, indicating that active influx is dominating over active efflux, has only been reported in very few investigations. The ratio of unbound AUC for the two enantiomers of gacyclidine in spinal cord compared with unbound AUC in plasma ranged between 3 and 7 (Hoizey et al., 2000). Sam et al. (1997) reported an unbound concentration ratio for the R (1/11002) and S (1/11001) enantiomer of apomorphine between brain ISF and blood of 12 and 5, respectively. This would indicate, also, that the net flux of apomorphine across the BBB is dominated by influx transport. Because only a few drugs have been investigated with methods that are able to discriminate between BBB transport and brain tissue binding in vivo, there are probably more examples of drugs that have a net influx across the BBB greater than 1. Genetic polymorphism has been described for P-glycoprotein (Kerb, 2006). Similar polymorphism in the transporter responsible for oxycodone BBB influx might have implications for individualized dosing, effective analgesia, and, perhaps, abuse.

The unbound CL in for oxycodone was 1910 μl/min · g brain, resulting in a rapid rate of equilibration across the BBB. This is even higher than the cerebral blood flow of 1440 μl/min · g brain (Shockley and LaManna, 1988). Recalculating CL in to the blood clearance of oxycodone across the BBB, CL in,blood, it was 1092 μl/min · g brain. This makes oxycodone a drug of high extraction into the brain. The CL in,blood for oxycodone is much greater than for morphine, morphine-6-glucuronide, and morphine-3-glucuronide of 11.4, 1.66, and 0.11 μl/min · g brain, respectively (Xie et al., 2000; Tunblad et al., 2003, 2005). However, it is in the same range as for fentanyl, with a value of 1840 μl/min · g brain from in situ brain perfusion studies (Dagenais et al., 2004). The CL in,blood for oxycodone was 630 μl/min · g brain. The interstitial bulk flow in rat brain has been reported to be 0.18 to 0.29 μl/min · g brain (Szentistvanyi et al., 1984), which makes the contribution of bulk flow to the total elimination of oxycodone from the rat brain negligible.

The terminology of BBB transport differs in the literature. The influx clearance across the BBB, CL in, in this study, can be compared with the initial brain uptake clearance in brain perfusion studies, remembering that the artificial fluid flow and composition in in situ brain perfusion differ from physiological blood flow. This will matter if the drug has a perfusion-limited transport across the BBB, which seems to be the case for oxycodone. To directly describe the equilibration across the BBB and thereby quantify the in vivo net result of active processes in the BBB, the extent of transport across the BBB is, in this study, described by K p,uu (Gupta et al., 2006). The term K p,uu includes both binding in brain tissue and protein binding in blood, and can therefore not be used to draw direct conclusions on BBB transport itself. K p,uu relates the unbound drug concentration in blood to the total concentration in brain. This gives information on the combined impact of transport across the BBB and distribution within the brain. The average K p,uu in this study was 7.9 ± 1.2, indicating that oxycodone is highly distributed into the brain. Because this can be the result of either extensive binding to brain tissue and/or influx or efflux mechanism at the BBB, it cannot be interpreted further without the use of unbound brain ISF concentrations. The V u,brain of 2.20 ml/g brain was larger than the brain interstitial space of 0.15 ml/g brain (Goodman et al., 1973). This shows that oxycodone is probably distributed intracellularly and/or binds to tissue components in the brain. Together
with the $K_{p,uu}$ ratio of 3, this shows that both binding in the brain tissue and influx mechanisms contribute to the high $K_{p,uu}$ value. Population modeling was applied to the data to obtain the typical values and RSEs of the pharmacokinetic parameters including the BBB parameters $CL_{in}$ and $K_{p,uu}$. The same type of integrated model has previously been used to describe morphine pharmacokinetics in the rat (Tunblad et al., 2004). The advantage of this integrated model is the possibility to estimate the pharmacokinetics, the BBB transport, and the blood and brain microdialysis probe recoveries simultaneously. The precision of the estimates was validated with the bootstrap resampling procedure, which showed a high degree of stability in the parameter estimates.

The insertion of a microdialysis probe into the brain region of interest is necessary to study the brain ISF pharmacokinetics of a drug. In the present study, the experiments were performed 24 h after surgery and probe implantation. The tissue trauma and the possible loss of BBB integrity associated with probe insertion are often discussed and could, if present, lead to errors in the interpretations of microdialysis data. Benveniste et al. (1987) showed that the local cerebral blood flow and the local cerebral glucose metabolism were nearly normalized 24 h after probe implantation. In contrast, Grothuis et al. (1998) showed that the BBB permeability was affected 24 h after probe insertion and that microdialysis may overestimate the rate of transfer into and out of the brain. However, many microdialysis studies in our and others’ hands showing an unbound brain to ratio far below unity are good evidence for a working experimental setup (Xie et al., 2000). With a very slow implantation of the microdialysis probes, we are confident that the results are describing an in vivo situation of a $K_{p,uu}$ above unity.

In conclusion, oxycodone was found to have 3 times higher unbound concentrations in the brain than in blood. The BBB influx clearance based on blood concentrations was close to brain blood flow, despite rather low lipophilicity. This indicates that oxycodone is actively influxed across the BBB in rats and might be an important keypoint when interpreting pharmacodynamic data from different routes of administration.

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