Morphine Blood–Brain Barrier Transport Is Influenced by Probenecid Co-Administration

Karin Tunblad,[1] E. Niclas Jonsson,[1] and Margareta Hammarlund-Udenaes[1,2]

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Purpose. The objective of this study was to investigate the possible influence of probenecid on morphine transport across the blood–brain barrier (BBB) in rats.

Methods. Microdialysis probes, calibrated using retrodialysis by drug, were placed into the striatum and jugular vein of seven Sprague–Dawley rats. Morphine was administered as a 4-h exponential infusion. The experiment was repeated the next day with the addition of probenecid, administered as a bolus dose (20 mg/kg) followed by a constant infusion (20 mg/kg/h). Models for BBB transport were built using the computer program NONMEM.

Results. The steady-state ratio of 0.29 ± 0.07 of unbound morphine concentration in brain to that in blood indicates that morphine is actively effluxed at the BBB. Probenecid co-administration increased the ratio to 0.39 ± 0.04 ($p < 0.05$). Models in which probenecid influenced the brain efflux clearance rather than the influx clearance, well described the data. The half-life in brain increased from 58 ± 9 min to 115 ± 25 min when probenecid was co-administered. Systemic clearance of morphine also decreased upon probenecid co-administration, and M3G formation was decreased.

Conclusion. This study indicates that morphine is a substrate for the probenecid-sensitive transporters at the BBB. Co-administration of probenecid decreased the brain efflux clearance of morphine.

KEY WORDS: blood–brain barrier; microdialysis; modeling; morphine; probenecid-sensitive transporters.

INTRODUCTION

Drugs of various physicochemical properties are efficiently excluded from the brain. This is partly because of the tightness of the blood–brain barrier (BBB) and also because of the presence of efflux proteins at the brain capillary endothelial cells that form the BBB. If passive diffusion is the only mechanism involved in transport of a compound at the BBB, the ratio of unbound drug concentrations between brain and blood at equilibrium would be unity, independently of the lipophilicity of the drug (1). Possible explanations for the occurrence of lower unbound concentrations in brain extracellular fluid (ECF) than in blood is active efflux transport, bulk flow of the brain ECF and/or metabolism in the brain.

Previous studies have demonstrated that morphine, which is a cation at physiologic pH, is a substrate for P-glycoprotein (Pgp) (2,3). It is currently unknown if any other transporters interact with morphine. Probenecid-sensitive transporters have been shown to be involved in transport across the BBB of morphine-3-glucuronide (M3G) (4). It has also been reported that pretreatment with probenecid potentiates the analgesic effect of morphine in rats (5). It is possible to increase exposure of the brain to drugs that are substrates for these transporters, and thus increase the effect of the drug, by co-administering probenecid.

Probenecid is known to inhibit several transporters. Among those are the multidrug resistance-associated proteins (MRPs), which act as organic anion transporters. MRPs have been shown to transport negatively charged compounds and glucuronide- and glutathione-conjugated compounds (6). However, it has also been shown that Tc-sestamibi (7), which is an organic cation, is a substrate for both Pgp and MRP in transfected cell lines, indicating an overlap in substrate specificity for Pgp and MRPs. Subtypes of the MRPs are located in the brain and also in other organs, such as the kidneys, the liver, and the gastrointestinal tract (8,9). In addition, transporters, such as organic anion transporting polypeptides (Oatp1 and Oatp2) and organic anion transporters (Oat1 and Oat3), which are expressed at the BBB, are inhibited by probenecid (10). In the present article, the term probenecid-sensitive transporters is used because the influence of probenecid on these and possibly other transporters at the BBB has not yet been clarified.

Microdialysis was used in this study because it has the advantage that samples can be taken continuously from different tissues *in vivo* without any loss of body fluids. Furthermore, microdialysis measures unbound drug concentrations, which are related to the pharmacological effect. In the rat, morphine is mainly eliminated by metabolism to the inactive metabolite M3G (11), but glomerular filtration and secretion of unchanged drug through the kidneys also occur (12). The objective of this study was to investigate the possible influence of probenecid on morphine transport across the BBB in rats.

MATERIALS AND METHODS

Animals

Seven male Sprague–Dawley rats (Møllegaard, Denmark) weighing 270–318 g were used. Before starting the experiments the rats were acclimatized for at least 7 days at 22°C. During this period the animals had free access to food and water. The Animal Ethics Committee of Uppsala University approved the protocol (C197/97).

Probes and Chemicals

The microdialysis probes used in the experiments were CMA/20 (10 mm; CMA, Stockholm, Sweden) and CMA/12 (3 mm) for measurements in blood and brain, respectively. The membrane of the probes had a cut off of 20,000 D. Morphine hydrochloride (10 mg/mL) and Enfluran® were purchased from the hospital pharmacy (Uppsala, Sweden). Sigma Chemicals (St Louis, USA) provided probenecid and low molecular weight heparin. The Ringer solution consisted of 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 0.2 mM ascorbic acid in 2 mM phosphate buffer with pH 7.4. All chemicals and solvents were of analytical grade.

[1] Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Biosciences, Uppsala University, Box 591, SE-751 24 Uppsala, Sweden.

[2] To whom correspondence should be addressed. (e-mail: Margareta.Hammarlund-Udenaes@farmbio.uu.se)

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Animal Surgery

The rats were anesthetized using inhaled Enfluran® (2.5%, balanced with 1.5 L/min oxygen and 1.5 L/min nitrous oxide). They were placed on a heating pad to maintain body temperature at 38°C during surgery. A PE-50 fused with a PE-10 was inserted into the femoral vein for drug administration, and into the femoral artery for blood sampling. The catheters were filled with a heparinized saline solution (100 IU/mL) in order to avoid clotting.

The blood probe (CMA/20) was perfused with a 0.1% low molecular weight heparin solution prior to implantation. Implantation into the right jugular vein was facilitated by the use of a guide cannula. The probe was then attached to the pectoral muscle via two sutures. For the insertion of the brain probe, the rat was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, USA), and a midline incision was made to expose the skull. A hole was drilled in the skull 2.7 mm lateral and 0.8 mm anterior to the bregma point. At these co-ordinates, and 3.8 mm ventral to the surface of the brain, a CMA/12 guide cannula with a dummy probe was implanted into the striatum and fixed to the skull by a screw and dental cement. The dummy probe was replaced with a CMA/12 probe when the cement had stiffened. A 20-cm piece of PE-50 tubing was looped subcutaneously on the back of the rat to the posterior surface of the neck, to let the perfusion solution reach body temperature before entering the brain probe. The ends of the cannulae were passed subcutaneously to the posterior surface of the neck and placed in a plastic cup sutured to the skin, out of reach of the rat. After surgery, the rat was placed into an individual CMA/120 system for freely moving animals, with free access to water and food. The animals were allowed to recover from the surgery for approximately 24 h.

Experimental Design

The experiment started with a stabilization period of 60 min, during which the probes were perfused with blank Ringer solution at a flow rate of 1 μL/min. During the stabilization period, four blank samples were collected and the infusion of a blank buffer solution (day 1) or a probenecid solution (day 2) was started. Probenecid was administered as a bolus dose of 20 mg/kg followed by a constant infusion of 20 mg/kg/h throughout the experiment.

The probes were calibrated *in vivo* according to the method of retrodialysis by drug (13) using a 100 ng/mL morphine solution in Ringer. The *in vivo* recovery was calculated by the loss of morphine from the perfusion solution to the surrounding tissue according to the following equation:

$$\text{Recovery}_{in\ vivo} = (C_{in} - C_{out})/C_{in} \quad (1)$$

Where C_{in} is the morphine concentration entering the probe and C_{out} is the concentration leaving the probe. The true unbound concentrations of morphine in brain ECF and venous blood were calculated from the dialysate concentrations after adjustment for the *in vivo* recovery. In total, five dialysate fractions were collected during the retrodialysis period. A washout period with blank Ringer solution was allowed for 90 min before starting the infusion of morphine. Blood gases were monitored, and a blank plasma sample was taken.

Morphine was administered as an exponential intravenous infusion over 4 h, aiming at reaching 1800 ng/mL in plasma instantaneously. The rate of the infusion was controlled via the STANPUMP computer-controlled infusion software (14). The pharmacokinetic parameters used by the software to calculate the infusion scheme were obtained from Ekblom *et al.* (15). Microdialysate fractions were collected every 10 min for the first 30 min after the start of the infusion and every 15 min until the end of the infusion. Dialysates were also collected at 10-min intervals during the first post-infusion hour and at 15-min intervals over the remaining hour. The dialysates were stored at –20°C until analysis. Arterial blood samples of 100 μL were collected at 0, 30, 60, 180, 235, 255, 270, 300, and 360 min. The plasma was separated by centrifugation for 5 min (7,200 g) and then frozen at –20°C until analysis.

The experiment was repeated on day 2. The only difference in methodology was the decapitation of half of the rats directly after the end of the morphine infusion. The brains from the decapitated rats were removed for analysis of total brain concentrations. They were divided into right and left hemisphere, and each part was weighed and stored at –20°C until analysis.

Monitoring of Blood Gas Status

The blood gas status (pH, pO_2, pCO_2, and O_2 saturation) of the rats was checked by injection of 25 μL arterial blood into an AVL Compact II blood gas analyzer (AVL Medical Nordic, Stockholm, Sweden). The respiratory parameters were monitored every hour up to 6 h after starting the morphine administration. Additional measurements were taken at 10, 30, and 270 min.

Drug Analysis

For the analysis of morphine in the dialysates, 13 μL was directly injected (Triathlon, Spark Holland, the Netherlands) into an high-performance liquid chromatography system. Separation was achieved using a Nucleosil C_{18} column (150 × 4.6 mm i.d. and 5-μm particles, Chrompack, Sweden). Morphine was detected using an electrochemical detector (Coulochem II, ESA Inc., Chelmsford, USA) with a guard cell (ESA 5020, ESA Inc.) and an analytical cell (ESA 5011, ESA Inc.). The detector potentials were set at 600 mV for the guard cell, and 0 mV and 450 mV for analytical cells one and two, respectively. For analysis of the blood dialysates, the mobile phase consisted of 580 ml 0.01 M phosphate buffer (pH 2.1), containing 0.4 mM sodium dodecyl sulphate (SDS), 420 mL methanol, and 20 mL tetrahydrofuran. The brain dialysates required a slightly modified mobile phase (620 mL phosphate buffer containing 0.4 mM SDS, 380 mL methanol, and 20 mL tetrahydrofuran). The mobile phase was delivered at 1 mL/min (ESA 580, ESA Inc.). The peak height was used for quantification of morphine. The standard curve was linear up to 500 ng/mL and the limit of quantification was 3 ng/mL (CV 5.9%).

Morphine concentrations in plasma were determined using the same chromatographic system as described above but with a potential of 300 mV for analytical cell 1. M3G in plasma was analyzed by fluorescent detection (Jasco 821-FP, Japan) at an excitation wavelength of 212 nm and an emission wavelength of 340 nm, coupled in series with the electrochemical detection. The mobile phase was changed to 670 mL 0.01 M phosphate buffer (pH 2.1), containing 0.2 mM SDS, 330 mL methanol, and 50 mL tetrahydrofuran. For each

sample, 100 μL of plasma was pretreated using a slightly modified method by Joel *et al.* (16). Morphine and M3G were eluted with 3 mL of methanol and evaporated under a stream of nitrogen at 45°C. The residue was dissolved in 150 μL of the mobile phase and 55 μL was injected into the high-performance liquid chromatography system. For morphine, the standard curve was linear up to 6000 ng/mL and the limit of quantification was 6 ng/ml (CV 5.1%). The limit of quantification for M3G was 101 ng/mL (CV 3.4%).

The total concentration of morphine in brain tissue was determined by homogenizing each brain hemisphere with a 5-fold larger volume of 0.1 M perchloric acid. The homogenates were centrifuged for 10 min at 600 g. Two hundred microliters of the supernatant was extracted according to the plasma method previously described.

Pharmacokinetic Analysis

The terminal half-life ($t_{1/2}$) of morphine in brain and blood was determined from the terminal rate constant (λ), which was obtained from log-linear regression of the terminal phase of the concentration-time curve. The equilibration ratio across the BBB was calculated from the unbound steady-state concentration ratio between brain and venous blood.

Pharmacokinetic Modeling

Unbound morphine concentrations were analyzed using nonlinear mixed effects modeling in the computer program NONMEM version VI (beta) (17) The analysis was conducted using the first-order conditional estimation method with interaction; population parameters, i.e., the model parameters, the magnitude of the residual errors and interindividual variability were assessed. The interindividual variabilities were modeled as being proportional to the corresponding parameter value. Model selection was based on graphical analysis using Xpose version 3.0 (18) and on the NONMEM objective function value (OFV). A decrease in the OFV of 3.84 between two nested models (1 degree of freedom) corresponds to $p < 0.05$. For the inclusion of probenecid as a categorical covariate, the required drop in the OFV was set to 3.84. Arterial concentrations were modeled after correction for protein binding (Fig. 1, model 1). Different nested models that were parameterized in terms of clearances and volumes were tested and probenecid was incorporated as a categorical covariate. The population parameter estimates from the plasma analysis were fixed and used in the analysis of the brain data.

Models with one or two brain compartments were investigated for the description of brain concentrations of morphine (Fig. 1, model 2). To define the pharmacokinetics in terms of CL_{in} as the influx clearance from blood to brain, and CL_{out} as the efflux clearance from the brain to blood for unbound morphine concentrations, the unbound volume of distribution for the brain was calculated using the following equation (19):

$$V_{u,\,brain} = (A_{br} - V_{bl} * C_{bl})/C_{u,br} \quad (2)$$

Where A_{br} is the total amount of morphine per gram of brain at steady state, V_{bl} is the volume of blood per gram of brain, C_{bl} is the total concentration in blood, and $C_{u,br}$ is the unbound brain concentration at steady-state. At a hematocrit of 44%, the blood to plasma ratio of morphine was 1.08 (20). The volume of blood in the rat brain has been estimated as 14 μL/g-brain (21) and A_{br}, C_{bl}, and $C_{u,br}$ were calculated from the rats that were decapitated on the second day of experiment (n = 3). With Equation (2), the volume was calculated as 2.8 ± 0.4 mL per brain (brain weight average 1.6 g), and this was used as a fixed value in the modeling. Because the amount of drug in the brain is small compared with the amount in the rest of the body, CL_{out} was assumed not to influence the systemic pharmacokinetics (Fig. 1, model 2). The differential equation system used to describe the mass balance of morphine in brain is expressed by Eqs. (3) and (4).

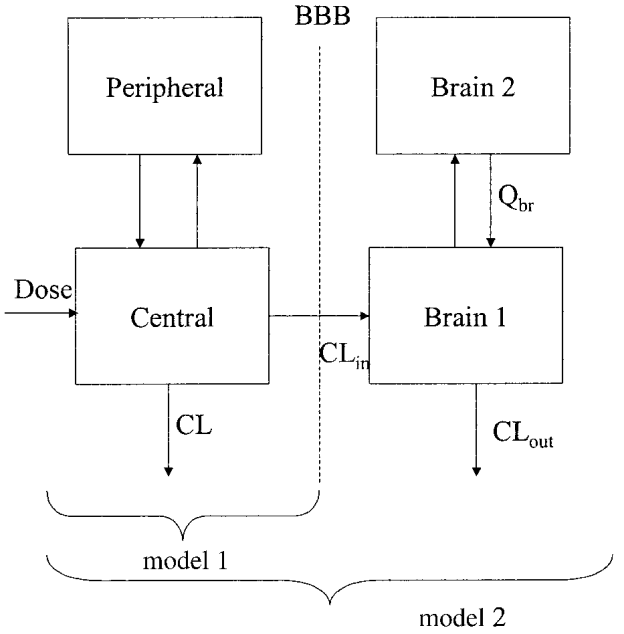

Fig. 1. Compartmental models for blood–brain barrier transport and brain distribution of morphine. Unbound arterial morphine concentrations were modeled first (model 1) and the population parameter estimates were used as the driving force for unbound brain concentrations (model 2). Abbreviations: *CL*, systemic clearance; CL_{in}, influx clearance from blood to brain; CL_{out}, efflux clearance from brain to blood; BBB, blood–brain barrier.

$$dC_{u,\,br1}/dt = (CL_{in} * C_{u,\,pl} + Q_{br} * C_{u,\,br2} - (CL_{u,\,out} + Q_{br}) * C_{u,\,br1})/V_{u,\,brain1} \quad (3)$$

$$dC_{u,\,br2}/dt = Q_{br} * C_{u,\,br1} - Q_{br} * C_{u,\,br2} \quad (4)$$

At steady state Equation 3 can be simplified to:

$$CL_{in}/CL_{out} = C_{u,ss,br}/C_{u,ss,bl} \quad (5)$$

During the model development, probenecid was tested as a covariate affecting either clearances or volumes.

Statistics

The pharmacokinetic parameters are presented as mean values and SDs. A two-sided paired *t*-test was used to differentiate between parameters (clearance, ratio of steady state concentrations, half-lives from brain and blood) from the two experimental days. The null hypothesis was rejected at a 5% significance level. The precision of the population parameters obtained from the NONMEM output was expressed as relative standard errors.

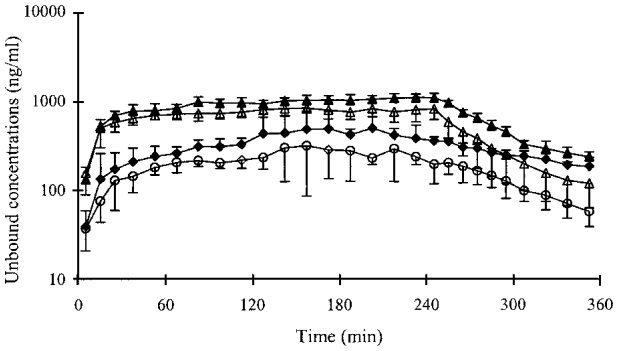

Fig. 2. Concentration–time profiles of unbound morphine (mean ± SD) in rat blood (triangles) and in brain extracellular fluid (diamonds) measured during a 4-h exponential intravenous infusion of morphine and 2 h after the end of the infusion. The open symbols show data where morphine only was administered, and the closed symbols show data where morphine and probenecid were co-administered.

RESULTS

The *in vivo* recovery of morphine was similar for the two experimental days for both probe types. Recoveries with the brain probe were 10.9 ± 1.5% and 10.5 ± 2.0% for day 1 and day 2, respectively. With the blood probe the recoveries were 47 ± 5.4% and 51 ± 7.7%, respectively.

Steady-state morphine concentrations in blood were reached quickly on day 1, as expected from the exponential infusion regimen (Fig. 2). The half-life in blood was extended by probenecid (37 min on day 1 vs. 52 min on day 2, $p < 0.05$; Table I). Thus, the time to reach steady-state in blood was somewhat delayed on the second experimental day. Probenecid influenced morphine systemic clearance resulting in a somewhat higher morphine steady state concentration day 2 (Table I). A two-compartment model, with probenecid as a covariate affecting systemic clearance, was best able to describe the plasma profiles of morphine. The typical value of morphine clearance was estimated to 28 mL/min after morphine administration, and 22 mL/min after the combined administration of morphine and probenecid ($p < 0.05$). Based on the area under the concentration-time curve (AUC) measurements of M3G, the formation of M3G from morphine was decreased by 41 ± 15% with probenecid co-administration.

Equilibrium in the brain was delayed in comparison with that in the blood, indicating slow transport of morphine into the brain (Fig. 2). The steady-state ratio for the unbound morphine concentration in brain ECF to that in venous blood was 0.29. When probenecid was co-administered, the steady state ratio was increased to 0.39 ($p < 0.05$; Table I). The terminal half-life was significantly longer in brain than in blood both with and without the co-administration of probenecid (Table I). During co-administration of probenecid the half-life of morphine in the brain increased from 58 min to 115 min ($p < 0.05$).

A two-compartment model was also required to describe the pharmacokinetics in the brain (Fig 1). Modeling of the BBB transport resulted in a good correlation between observed and predicted concentrations over time (Fig. 3). The ratio between CL_{in} and CL_{out} from the modeling is equivalent to the ratio of brain ECF to unbound blood concentrations at steady-state, and the modeling gave similar ratios for brain ECF to unbound arterial concentrations of 0.27 and 0.39 on the two days (Table II). The inclusion of probenecid as a covariate affecting the efflux clearance improved the correlation between observations and predictions substantially, and resulted in a drop in the OFV of 107. The improvement was much smaller if probenecid was affecting the influx clearance or the intercompartmental clearance. The influx clearance was estimated as 11.4 μL/min*g-brain on both days and the efflux clearance was estimated as 42 μL/min*g-brain without co-administration of probenecid and 29 μL/min*g-brain with probenecid (Table II). The intercompartmental clearance in the brain (Q_{br}) of 94 μL/min*g-brain was greater than the efflux clearance (Table II).

Only small changes in pH, pCO_2, and O_2 saturation were observed during the morphine infusion on both experimental days. However, there was a significant decrease in pO_2 from 12.4 kPa at baseline to an average minimum of 8.1 kPa ($p < 0.05$) at 180 min on day 1. The same result was observed on day 2, with an average minimum value of 9.6 kPa (at 180 min), which was lower than the baseline value of 13.2 kPa ($p < 0.05$).

DISCUSSION

The significant increase in the brain to blood ratio of unbound morphine from 0.29 to 0.39 after probenecid administration indicates that morphine is a substrate for probenecid-sensitive transporters situated at the BBB. Although significant, the size of the increase to a value substantially smaller than unity suggests that probenecid-sensitive transport of morphine is not of major importance in excluding morphine from the brain. The reason is that if the probenecid-sensitive transporters were the only transporters involved in morphine brain efflux, the brain to blood ratio of morphine should have been unity; apart from the bulk flow contribution, upon probenecid co-administration. It has previously been shown that morphine is also a Pgp-substrate (2,3), which together with our present findings indicate that more than

Table I. Half-Lives, Unbound Steady-State Concentrations in Blood ($C_{u,\ ss,bl}$) and Unbound Steady-State Ratios of Morphine in Brain and Blood with and without Probenecid Co-Administration (Mean ± SD)

	Half-life (min)			$C_{u\ ss,bl}$	Ratio
	Brain ng/mL	Venous blood	Arterial blood	venous blood	$C_{u\ ss,br}/C_{u\ ss,bl}$
Morphine	58 ± 9[a]	37 ± 5	42 ± 9	779 ± 156	0.29 ± 0.07
Morphine + Probenecid	115 ± 25[a,b]	52 ± 15[b]	57 ± 11[b]	1058 ± 116[b]	0.39 ± 0.04[b]

Note: Values were determined non-compartmentally.
[a] $p < 0.05$ vs. venous and arterial blood.
[b] $p < 0.05$ vs. morphine only.

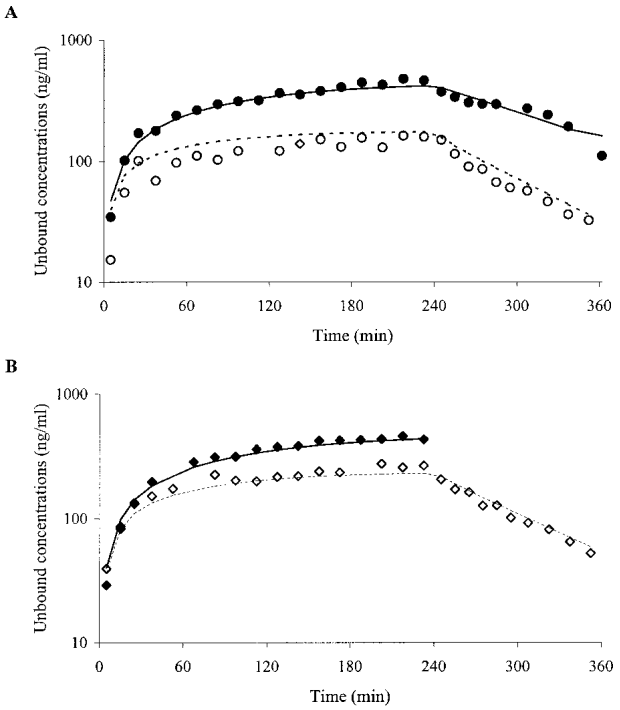

Fig. 3. Observed (diamonds) and predicted (lines) unbound morphine concentrations in the brain extracellular fluid over time in two representative rats. Morphine concentrations were measured during a 4-h exponential infusion of morphine and 2 h after the end of the infusion. A shows one rat that was followed during the whole experiment on both experimental days, and B shows another rat that was decapitated on the second day of experiment. The open symbols and dashed lines represent the data where morphine only was administered, and the closed symbols and the solid line the data where morphine and probenecid were co-administered.

one transporter is active in morphine brain efflux. Another example is methotrexate, which was reported to be a substrate for both Pgp and MRP (22). In contrast, M3G is transported by the probenecid-sensitive transporters and not by Pgp (2,4).

From the pharmacokinetic modeling of brain concentrations, probenecid was found to decrease morphine efflux clearance from the brain. Morphine CL_{in} was estimated as 11.4 μL/min*g-brain and CL_{out} as 42 μL/min*g brain, which is close to the values reported by Bouw *et al.* (23). With probenecid co-administration, the CL_{out} was 29 μL/min*g-brain. The contribution of the bulk flow to the total elimination of morphine from the rat brain is likely to be small, as the interstitial bulk flow from the rat brain is reported to be 0.18–0.29 μL/min*g-brain (24).

The unbound volume of distribution in the brain for morphine at steady-state of 1.7 mL/g-brain was larger than the brain extracellular space of 0.15–0.20 mL/g-brain tissue (25). This suggests that morphine penetrates into cells and/or binds to the brain tissue. The central volume of distribution in the brain was estimated as 0.14 mL/g-brain tissue.

Probenecid also affected the pharmacokinetics of morphine in blood by decreasing its systemic clearance. In the rat, metabolism to M3G is the dominant route of morphine elimination. No morphine-6-glucuronide (M6G) is formed and renal excretion accounts for only 20% of the total elimination of morphine (11). The effect of probenecid on morphine metabolism to M3G was clearly observed in the AUC of M3G in plasma. The change in AUC of M3G was explained by decreased metabolite formation, as no effect of probenecid on the elimination of M3G was found in an earlier study (4). Inhibition of glucuronidation has also been shown for ketoprofen (26) and zomepirac (27) when these drugs were co-administered with probenecid.

It has been shown that uridine diphosphate glucuronosyltransferase is responsible for morphine glucuronidation in the rat (11), and that there is no interaction between uridine diphosphate glucuronosyltransferase and morphine in the rat brain (28). Therefore, any influence of probenecid on possible M3G formation in the brain is unlikely. The increase in morphine AUC in the brain was greater than the increase observed in blood, indicative of a more pronounced effect of probenecid on morphine transport across the BBB than on its elimination from the body.

The doubling of the half-life in the brain on day 2 suggests that the rate-limiting step for transport of morphine out of the brain is the actual transport across the BBB rather than redistribution within the brain. Co-administering morphine with the potent Pgp inhibitor GF120918 also resulted in an increased half-life of morphine in the brain (3).

In this study probenecid was shown to influence the active transport by increasing the brain to blood AUC ratio of morphine. However no change in the *in vivo* recovery was observed. According to the theory of quantitative microdialysis, the probe recovery depends on the resistances from the dialysate, the membrane and the external medium, i.e., the tissue surrounding the microdialysis probe (29). The resistance of the external medium is usually the most important contributor to the overall resistance for a solute. Both passive diffusion and active transport affect this resistance and sub-

Table II. Population Parameter Estimates for the Blood–Brain Barrier Transport of Morphine Expressed as Typical Values (Relative Standard Error [RSE %])

	Population estimates (RSE %)						
	CL_{in} (μL/min*g-brain)	IIV (%)	CL_{out} (μL/min*g-brain)	IIV (%)	CL_{in}/CL_{out}	V_{brain1} (mL/g-brain)	Q_{br} (μL/min*g-brain)
Morphine	11.4 (9)	13	42 (10)	NE	0.27	0.14 (91)	94.4 (25)
Morphine + Probenecid	11.4 (9)	13	29 (38)[a]	NE	0.39[a]	0.14 (91)	94.4 (25)

Note: CL_{in}, influx clearance from blood to brain extracellular fluid; IIV, interindividual variability; CL_{out}, efflux clearance from brain extracellular fluid to blood; CL_{in}/CL_{out}, ratio between influx clearance and efflux clearance; V_{brain1}, central volume of distribution in brain; Q_{br}, brain intercompartmental clearance and NE, not estimated.
[a] $p < 0.05$ vs. morphine only.

sequently the *in vivo* recovery (30). The unaffected recovery indicates that the contribution of the active process to the total resistance is relatively small and/or the probenecid-sensitive transporters are of minor importance for the tissue resistance for morphine. In conclusion, this study indicates that morphine is a substrate for the probenecid-sensitive transporters at the BBB, and that probenecid decreases the brain efflux clearance of morphine.

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