Blood-Brain Barrier Equilibration of Codeine in Rats Studied with Microdialysis

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Purpose. The purpose of the study was to investigate the distribution of codeine across the blood-brain barrier (BBB) in rats by microdialysis (MD).

Methods. Rats were administered intravenous infusion of codeine in doses of (1) 10 mg/kg, (2) 20 mg/kg for 10 min, and (3) an exponential infusion for 2 h aiming at a plasma concentration of 2500 ng/ml, in a crossover design (n = 6). Microdialysis was used to determine codeine unbound concentrations in blood and brain extracellular fluid (ECF). Total brain tissue and plasma concentrations were also determined. Nalorphine was used as a calibrator for measurement of in vivo recovery.

Results. Relative recovery and retrodialysis loss of codeine and nalorphine were similar both in vitro and in vivo. Codeine was rapidly transported into the brain ECF with identical influx and efflux clearance across the BBB. The AUC ratios of brain to blood were 0.99 ± 0.25 and 0.95 ± 0.16 for Dose 1 and 2, respectively. The C_{ss} ratio of brain to blood was 1.06 ± 0.12 for the exponential infusion. The half-lives were 25 ± 4 min, 22 ± 2 min in blood and 27 ± 5 min, 25 ± 5 min in brain for Dose 1 and Dose 2, respectively. Total brain tissue concentrations were 3.6 ± 1.2-fold higher than the unbound concentrations in brain. Codeine was demethylated to morphine with an unbound $AUC_{blood,morphine}/AUC_{blood,codeine}$ ratio of 7.7 ± 5.1% in blood. No morphine was detected in brain MD, but total concentrations were possible to measure.

Conclusions. Codeine rapidly reached a distributional equilibrium with equal unbound concentrations in blood and brain. The brain transport of codeine did not show any dose-dependency.

KEY WORDS: microdialysis; codeine; morphine; blood-brain barrier; pharmacokinetics.

INTRODUCTION

Codeine has been commonly used for a long time as an analgesic drug. As a highly lipophilic substance (olive oil/water partition coefficient 0.16 (1), octanol/water partition coefficient 3.98 (2), codeine should be rapidly and completely transported across the blood-brain barrier (BBB). At equilibrium, equal unbound concentrations should be reached on both sides of the membrane if no active transport mechanisms are present (3, and others). It was early proven by brain uptake index (BUI) and brain homogenate methods (4,5) that codeine was rapidly transported into the brain. Unbound concentrations has not earlier been possible to measure.

The microdialysis technique has made it possible to measure unbound concentrations in blood and tissues over time in conscious animals (6). Therefore, the technique can give new insights into BBB equilibration (3). Moreover, microdialysis can be used in crossover studies in the same animal, which cannot be achieved with other methods (7,8).

After carotid artery injection the amount taken up by the brain is almost entirely a function of BBB permeability (9). By 30 sec after an intravenous injection more than 90% of codeine has left the blood, and the maximum concentration of codeine in brain was reached by 1 to 4 min (4). The plasma protein binding of codeine is low (7–25%) (2). Total brain codeine concentrations were reported to be higher than plasma concentrations by a factor of 2–5 at different time points after i.p. or s.c. administration (10,11). The codeine uptake ratio in cerebral cortical slices to medium in vitro was 7 after 10 min incubation at 37°C, and codeine was transported into the brain slices by an active transport system (12).

The driving force for transport across the BBB is the unbound concentrations in blood and brain extracellular fluid (ECF). BBB structure and physio-chemical properties of the drug, and also plasma protein binding, will influence the permeability of the drug.

The primary objective of this study was to investigate the distribution characteristics of unbound codeine across the BBB in rats by microdialysis in a crossover design. The formation of morphine from codeine was also studied.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Sweden) weighing 280–320 g were used. The rats were group housed at 22°C under 12 h light-dark cycle for at least one week before the experiment. Food and water were available ad libitum. Ethical approval was obtained from the Animal Ethics Committee of Uppsala University.

Chemicals

Codeine phosphate and morphine hydrochloride were obtained from the hospital pharmacy (Uppsala, Sweden). Nalorphine chloride was provided by Sigma (St. Louis, USA). Hypnorm® was purchased from Janssen Pharmaceutics (Beerse, Belgium). 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, pH 7.4. All chemicals were of analytical grade. Solvents were of HPLC grade.

Probes

Microdialysis probes, CMA/12 (3 mm, 400 μm i.d., 500 μm o.d.) and CMA/20 (10 mm, 500 μm i.d., 670 μm o.d.), were used as the sampling devices for measuring the striatum and blood concentrations of codeine and morphine, respectively. The probe membranes have a 20000 daltons molecular weight cutoff.

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Animal Surgery

The rats were intramuscularly anaesthetised with Hypnorm® (1 ml/kg). The 30 cm PE-50 cannulae were fused with 2 cm PE-10 and filled with saline containing 200 EU Heparin to prevent clotting. The PE-10 ends were inserted into the left femoral artery and vein in order to collect blood samples and to administer codeine, respectively. The blood probe (CMA/20, 10 mm) was perfused with 0.1% low molecular weight heparin solution before it was inserted into the right jugular vein via a guide cannula and then fixed with two sutures. The anaesthetised rat was then placed on the stereotaxic instrument (David Kopf Instruments, Tujunga, USA) and a midline incision was made to expose the skull. A CMA/12 guide cannula was implanted into the striatum with coordinates: lateral 2.7 mm, anterior 0.8 mm, ventral 3.8 mm relative to bregma. The guide cannula was fixed to the skull by a screw and dental cement, over which the skin was sutured. A 20 cm PE-50 tubing was looped subcutaneously distal to the posterior surface of the neck, allowing the perfusion solution to reach rat body temperature before it entered the CMA/12 probe. The protruding ends of all cannulae were passed subcutaneously to the posterior surface of the neck, and were protected by a plastic cap sutured to the skin. The animals were allowed to recover for 24 hours.

Experimental Design

Calculation of Recovery In Vitro

To validate nalorphine as a calibrator to codeine in vivo, both CMA/12 (n = 3) and CMA/20 (n = 3) probes were used to determine microdialysis relative extraction from the surrounding medium to the probe, relative recovery (RR), and relative extraction from the probe dialysate to the medium, retrodialysis loss (RD) in vitro. According to the situation in vivo, the recovery experiments were performed at 37°C. The probes were first placed in 1 ml Ringer medium containing codeine (500 ng/ml). The perfusion Ringer solution contained nalorphine as a calibrator (250 ng/ml). Then the probes were placed in 1 ml blank Ringer solution as the medium, and the perfusate contained codeine (500 ng/ml) and nalorphine (250 ng/ml). The probes were perfused at 2 μl/min with a CMA/100 Microinjection Pump. The microdialysis samples were collected automatically by a CMA/140 Microfraction Collector (Carnegie Medicine, Stockholm, Sweden) for 70 min at 10 min intervals. The calculation of recovery was based on Equation (1):

$$\text{Recovery}_{\text{in vitro}} = \left(\frac{C_{out} - C_{in}}{C_m - C_{in}}\right) \quad (1)$$

where C_{out} is the substance concentration in the outflow, C_{in} is the substance concentration in the inlet and C_m is the substance concentration in the surrounding medium.

Preparation for the Experiment

On the first day of microdialysis, the dummy probe was removed from the guide cannula and a CMA/12 probe was inserted into striatum. The CMA/20 and CMA/12 probes were perfused with Ringer solution for 1 h to stabilize the system and to obtain blank samples. After the blank period, a retrodialysis period was started by changing the perfusion solution to Ringer containing codeine (500 ng/ml), and nalorphine (250 ng/ml) for 70 min. Thereafter, a washout period followed for 60 min when the perfusate was changed to one containing only nalorphine. Microdialysis samples were collected at 10 min intervals.

Calculation of Recovery In Vivo

Microdialysis recovery in vivo was calculated in each rat by continuous retrodialysis throughout the experiments, with nalorphine as the calibrator. The in vivo recovery of codeine was determined by the loss of nalorphine and the ratio of retrodialysis recovery of codeine and nalorphine:

$$\text{Recovery}_{\text{in vivo}} = \left(1 - \frac{C_{out,\,nal}}{C_{in,\,nal}}\right) * \frac{\text{Codeine}_{\text{RD in vivo}}}{\text{Nalophine}_{\text{RD in vivo}}} \quad (2)$$

Administration Procedure of Codeine

Six rats were used in the experiments with a randomized crossover design over two days. Each animal received three doses, a 10 min intravenous infusion of 10 mg/kg (24.6 μmol/kg, Dose 1), and 20 mg/kg (49.2 μmol/kg, Dose 2) codeine on one day, and an exponential infusion for 2 hr to rapidly reach the target plasma concentration of 2500 ng/ml using the Stanpump CCI system (13,14) on the other day (Dose 3). The pharmacokinetic parameters for Stanpump were taken from reported values (15). The rats receiving the exponential infusion on Day 2 were decapitated immediately after stopping the infusion, and whole brain tissue was collected and frozen at −20°C until analysis for total brain concentrations. Arterial blood (100 μl) was drawn at 0, 9, 15, 90, 120, 125, 129, 135 and 210 min for the 10 min infusions, and at 0, 10, 60, 120 min for the exponential infusion. The plasma was separated by centrifugation (10000 rpm, 5 min) and frozen at −20°C until analysis. Microdialysis samples were collected at 5 min intervals for the first 30 min, and at 10 min intervals thereafter, and frozen at −20°C until analysis. The perfusion through the MD probes were continued over night with blank Ringer at a flow rate of 0.5 μl/min. The flow rate was switched back to 2 μl/min on the second experimental day, and the recovery procedure was repeated before the start of the experiment.

Sample Analysis

Microdialysis Samples

Seventeen μl of the MD samples were directly injected into the Nucleosil C_{18} HPLC column (5 μm particles, 4.6 × 150 mm, Netherlands). The samples were diluted with 10 μl for the 5 min sampling intervals. An ESA Coulochem electrochemical detector (Model 5100A, ESA, Inc. Massachusetts, USA) with guard cell 5020 and analytical cell 5011 was used for the morphine and nalorphine analyses. The Coulochem detector potentials were set at 600, 0 and 450 mV, for guard cell, cell 1 and cell 2, respectively. Codeine was analyzed by UV detection (Shimadzu SPD-10A, Japan) at a wavelength of 223 nm, coupled in series with the electrochemical detector. The flow rate was 1 ml/min, and the mobile phase consisted of 650 ml 0.01 M phosphate buffer (pH 2.1) containing 0.2 mM sodium dodecyl sulphate, 350 ml methanol and 20 ml

tetrahydrofuran. An integrator with two channels (Shimadzu C-R5A, Japan) was used.

Plasma Samples

The plasma samples were extracted with Sep-Pak C_{18} cartridges (Waters), which were first activated with 5 ml methanol, 3 ml 0.01 M phosphate buffer (pH 2.1) and 5 ml distilled water filtered through the cartridge under vacuum in order. Plasma (100 μl) was mixed with 3 ml of 0.5 M ammonium sulphate buffer (pH 9.3) in a 10 ml polystyrene tube for 5 sec, and transferred to the reservoir. The plasma samples were filtered through the cartridges which were subsequently washed with 20 ml 0.005 M ammonium sulphate buffer (pH 9.3), 0.5 ml distilled water and 0.1 ml methanol under vacuum. Lastly, 3 ml methanol was added and the eluates were collected and evaporated under a stream of nitrogen at 45°C. The dried residues were reconstituted in 150 μl mobile phase, of which 50 μl was injected onto the HPLC column. The analytical method was the same as that described for the MD samples, except that the Coulochem detector potential for cell 1 was 300 mV.

The limit of quantification of codeine and morphine in MD samples were 50 ng/ml and 4 ng/ml, with coefficient of variation (CV) of 4.8% and 4.9% at these limits, respectively. The limit of quantification of codeine and morphine in plasma were 50 ng/ml and 6 ng/ml, with CV's of 6.4% and 7.1% at 150 ng/ml and 18 ng/ml, respectively. The absolute extraction recoveries for plasma samples of codeine and morphine were between 88% and 97%.

Brain Tissue

The brain tissue was homogenized with a 5-fold volume of 0.1 M perchloric acid and then centrifuged for 20 min at 10000 rmp. One hundred μl of the supernatant was extracted in the same way as the plasma samples. The analytical method was the same as for plasma with a modified mobile phase consisting of 665 ml phosphate buffer, 335 ml methanol and 20 ml tetrahydrofuran. The limit of quantification of codeine and morphine were 143 ng/ml and 8 ng/ml, with CV's of 6.5% and 6.6% at 476 ng/ml and 40 ng/ml, respectively. The absolute recoveries for brain tissue of codeine and morphine were between 88% and 101%.

Data Analysis

Calculation of Concentrations in Brain ECF and Blood

The unbound concentrations were calculated from dialysate concentrations corrected by the recovery in vivo (Eq. 2) at each collection interval.

Pharmacokinectic Analysis

The terminal rate constants of codeine in blood and striatum were estimated from the last nine observed MD data points. The areas under the concentration-time curves of unbound drug (AUC_u) in blood and brain were calculated by the linear trapezoidal method and the residual areas to infinity were obtained by C_n/λ, where C_n is the concentration of the last sample, and λ is the rate constant.

The unbound body clearance (CL_u) was calculated from the ratio of dose and AUC_u. The unbound volume of distribution ($V_{d,u}$) was determined by the ratio of CL_u and λ.

Paired student's *t*-test was used to compare differences between half-lives and AUC_u ratios. A value of $p < 0.05$ was considered to be significant. All data are presented as mean ± SD.

RESULTS

Codeine and nalorphine had similar microdialysis properties in vitro. For the 10 mm CMA/20 probes, RR and RD of codeine and RD of nalorphine were 50.5±1.6, 49.8 ± 4.9, and 49.3 ± 3.3, respectively. For the 3 mm CMA/12 probes, RR and RD of codeine and RD of nalorphine were 24.4 ± 1.8, 24.7 ± 2.0, and 24.9 ± 1.7, respectively. The recovery ratio in vitro of codeine and nalorphine was 0.98 ± 0.03 for CMA/12, and 1.0 ± 0.02 for CMA/20.

The in vivo recoveries are exhibited in Table I. There were no statistical differences between the RD of codeine and nalorphine for blood and brain probes within or between days.

The unbound concentration-time profiles of codeine in blood and striatum were superimposable (Fig. 1), with an AUC_u ratio brain:blood of 1.0 ± 0.2 independent of dose (Table II). The brain showed a tendency towards a more rapid increase in concentrations than the venous blood during the exponential infusion (Fig. 1b), which is to be expected for a lipophilic compound that is easily transported across the BBB.

The terminal half-lives were similar in blood, 24 min, and brain, 26 min (Table II). There was no dose-dependency or treatment order effect in the disposition kinetics of codeine. The AUC_u ratios between Dose 2 to Dose 1 were 2.3 ± 1.0 and 2.4 ± 1.3 in blood and brain, respectively. The unbound body clearance was 4.7 ± 1.2 L/h*kg, and the unbound apparent distribution volume was 2.7 ± 0.8 L/kg.

Unbound morphine was detected in the blood MD sample at 7.5 min and thereafter (Fig. 2). The half-lives of unbound morphine in blood were longer than for codeine, and 40 ± 8 min and 39 ± 9 min, respectively, for Dose 1 and Dose 2. Maximal concentrations of 124 ± 65 ng/ml and 212 ± 63 ng/ml were reached at 22.5 min and 17.5 min, respectively, for

Table I. Comparison of Retrodialysis Recovery of Codeine and Nalorphine *In Vivo*

Probe (n = 6)	$RD\%_{1st}$[a] codeine	$RD\%_{1st}$[a] nalorphine	$Ratio_{1st}$[b] cod/nal	$RD\%_{2nd}$[c] codeine	$RD\%_{2nd}$[c] nalorphine	$Ratio_{2nd}$[d] cod/nal
CMA/20	32.1 ± 11.8	33.9 ± 14.0	1.0 ± 0.1	36.5 ± 8.5	33.0 ± 8.9	1.1 ± 0.3
CMA/12	8.6 ± 3.2	8.5 ± 3.4	1.0 ± 0.2	8.0 ± 1.8	8.0 ± 2.3	1.0 ± 0.2

[a] Retrodialysis loss during the first day.
[b] The ratio of retrodialysis loss of codeine to nalorphine during the first day.
[c] Retrodialysis loss during the second day.
[d] The ratio of retrodialysis loss of codeine to nalorphine during the second day.

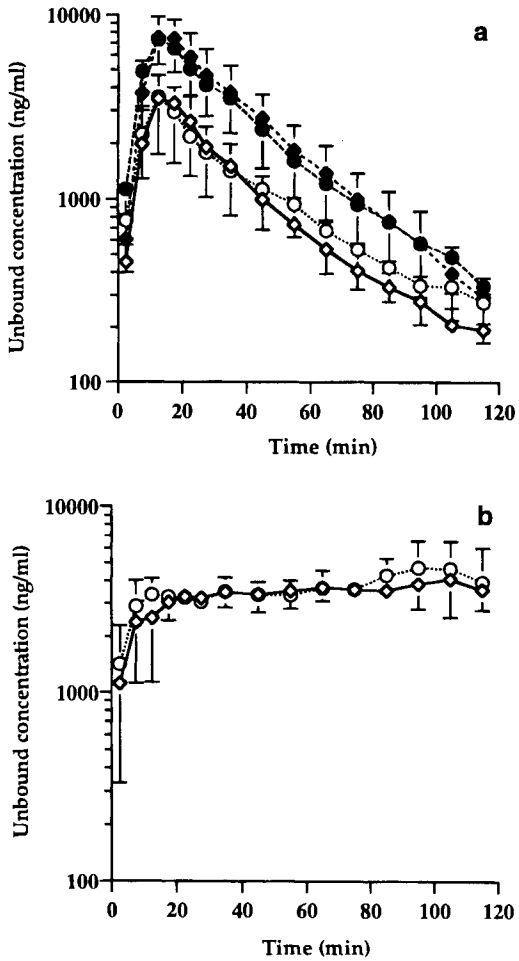

Fig. 1. (a) The concentration-time curves of codeine in blood (◇) and striatum (○) following a 10 mg/kg iv infusion for 10 min, and in blood (◆) and striatum (●) following a 20 mg/kg iv infusion (n = 6), (b) The concentration of codeine in blood (◇) and striatum (○) versus time profile after the exponential infusion for 2 h aiming at a concentration at 2500 ng/ml (n = 6). No significant difference between $C_{u,ss,blood}$ and $C_{u,ss,brain}$.

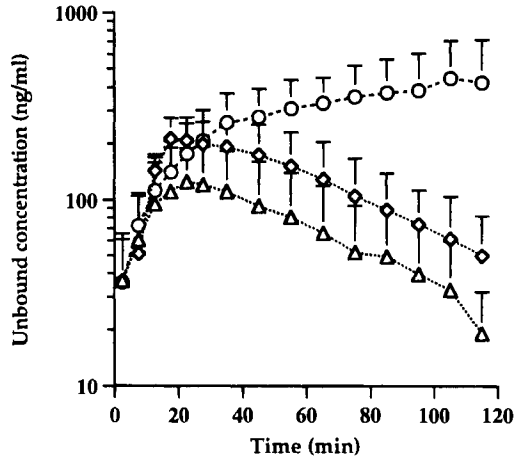

Fig. 2. The concentration-time profiles of unbound morphine in blood after codeine administration of 10 mg/kg (△), 20 mg/kg (◇), and exponential infusion (○) (n = 6).

Dose 1 and Dose 2. The AUC_u ratios of morphine to codeine were 7.8 ± 7.0%, 7.6 ± 5.8%, and 7.8 ± 3.3%, after Dose 1-3, respectively. No morphine was detected in the brain MD samples except in one rat at a few time points. This implies that unbound morphine concentrations were below 4 ng/ml in brain.

During the codeine infusion, the unbound venous concentrations of codeine and morphine were lower than the total concentrations in the femoral artery, whereas postinfusion levels were higher in the veins, although parallel, which is to be expected for a highly extracted drug (Fig. 3). The AUC_u of codeine in MD sampled venous blood was higher than that in sampled arterial plasma but, due to sparse sampling of arterial blood, the exact values were not calculated. During the exponential infusion, there was no significant difference ($p > 0.05$) between the MD determined unbound C_{ss} in venous blood (3436 ± 304 ng/ml, n = 6) and total C_{ss} in arterial plasma (2955 ± 566 ng/ml, n = 6).

In brain, the ratio between total and unbound codeine concentration was 3.6 ± 1.2 at the end of exponential infusion (Table III). The total concentration ratio of codeine between

Table II. Parameters for Blood-brain Barrier Distribution and Pharmacokinetics of Codeine for a Crossover Study with Infusions over 10 min (10 and 20 mg/kg) and an Exponential Infusion over 2 Hours (n = 6)

Dose (mg/kg)	$t_{1/2,blood}$[a] (min)	$t_{1/2,brain}$[a] (min)	$AUC_{u,blood}$[b] (μg*min/ml)	$AUC_{u,brain}$[b] (μg*min/ml)	$AUC_{u,br}/AUC_{u,bl}$	CL_u[c] (L/h*kg)
10	25.3 ± 4.0	27.3 ± 4.7	167 ± 10	161 ± 85	0.99 ± 0.25	4.85 ± 0.93
20	22.4 ± 1.9	24.6 ± 5.2	293 ± 91	278 ± 93	0.95 ± 0.16	4.43 ± 1.59
exp[d]	—	—	3435 ± 304[e]	3641 ± 539[f]	1.06 ± 0.12[g]	—
Average	23.9 ± 3.3	26.0 ± 5.0	—	—	1.00 ± 0.18	4.66 ± 1.22

[a] Terminal half-life for codeine.
[b] Area under the unbound concentration-time curve.
[c] Unbound body clearance.
[d] Exponential infusion aiming at a concentration of 2500 ng/ml based on data from ref. 15.
[e] Steady-state concentration in blood (ng/ml).
[f] Steady-state concentration in brain (ng/ml).
[g] Ratio of steady-state concentrations between brain and blood.

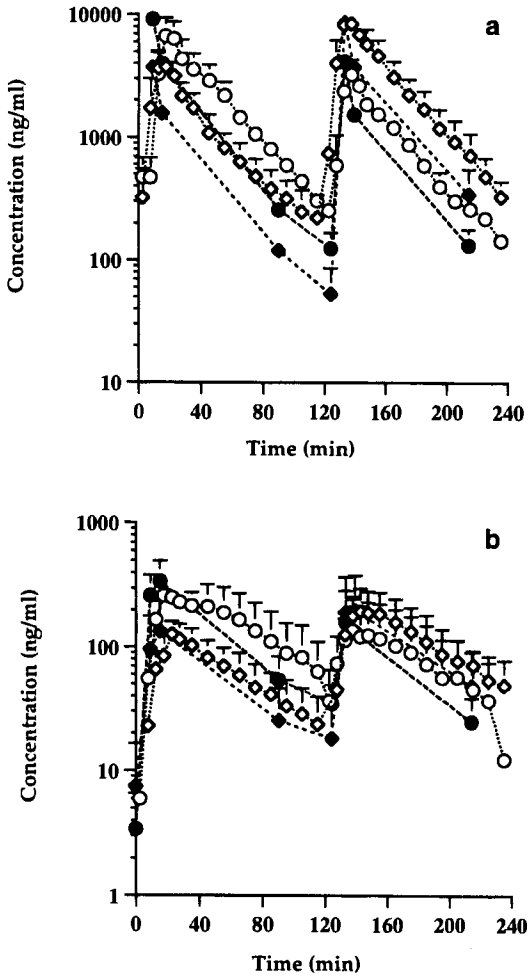

Fig. 3. (a) Codeine and (b) morphine concentration-time curves (n = 3) after codeine administration: plasma (♦) and blood MD (◇) 10 mg/kg following 20 mg/kg, plasma (●) and blood MD (○) 20 mg/kg following 10 mg/kg.

Table III. Unbound and Total Concentrations of Codeine and Morphine in Blood and Brain at the End of the Exponential Infusion During the Second Day (n = 3)

	Blood (ng/ml)			
	Unbound (venous MD)	Total (arterial plasma)	$Ratio_{tot/u}$[a]	
Codeine	3567 ± 81	3478 ± 822	0.97 ± 0.22	
Morphine	475 ± 224	506 ± 248	1.06 ± 0.32	
	Brain (ng/ml)			
	Unbound	Total	$Ratio_{tot/u}$[a]	$Ratio_{tot\ br/tot\ pl}$[b]
Codeine	3811 ± 325	13955 ± 5666	3.60 ± 1.16	3.98 ± 0.98
Morphine	—	292 ± 205	—	0.58 ± 0.20

[a] The concentrations ratio of total to unbound.
[b] The total concentration ratio of brain to arterial plasma.

brain and blood was 4.0 ± 1.0. The ratio of total morphine concentrations between brain and blood was 0.6 ± 0.2.

DISCUSSION

The present study was designed to investigate the BBB transport of codeine in conscious rats with the microdialysis technique. Codeine is a lipophilic drug within the opioid group and is theoretically expected to show similar unbound concentrations at both sides of the BBB.

The key issue in measuring unbound concentration in tissues by microdialysis is a correct estimation of recovery in vivo. It is well-known that in vivo recovery cannot be replaced by in vitro recovery (16,17). Validation of nalorphine as a calibrator to determine codeine recovery in vivo was included in this study. The in vitro and in vivo results demonstrate that codeine and nalorphine have similar microdialysis properties, and that the retrodialysis loss of nalorphine was equal to that of codeine in vivo. These results suggested that nalorphine was reliable as a calibrator for estimation of in vivo recovery by retrodialysis.

Codeine was rapidly transported into the brain with equal striatal and blood unbound concentration-time profiles. The AUC ratio of brain ECF to blood was 1.0 ± 0.2, so the influx clearance from blood to brain, Cl_{in}, was identical to the efflux clearance from brain to blood, CL_{out}. This suggests that only passive processes participate in codeine BBB transport. During the exponential infusion there was a tendency that codeine more quickly reached the steady-state concentration in brain than in blood. As codeine is a highly lipophilic drug, its penetration primarily depends on the permeability of the blood-brain barrier and blood flow (5). Oldendorf noted that when P value (olive oil/water partition coefficient) was greater than about 0.03, a substantial fraction of the drug penetrated the BBB, and uptake was essentially complete(1). The codeine concentrations in brain increased somewhat during the last 40 min of the infusion, which could not be explained.

In this study, the total codeine concentrations in brain homogenates was about 3.6-fold higher than the unbound concentration in brain and 4-fold higher than total in plasma concentration, which is close to earlier published data (10,11). Comparison of total brain to plasma concentrations cannot completely elucidate the transport equilibrium across the BBB, as the total concentrations include binding processes both to the proteins in blood and to brain components. When the drug has a high tissue affinity, the brain homogenate method might underestimate efflux transport from brain, as the concentration in brain tissue is higher than the unbound in brain ECF (18).

As codeine is highly taken up by brain tissue and has a short half-life, this may contribute to the arterio-venous differences observed. In theory, the AUC or steady-state plasma concentrations should be the same between arterial and venous blood, but there are many reported exceptions (19,20). In the present study, the arterial AUC was smaller than the unbound venous MD AUC. However, at steady-state, the ratio was unity.

Codeine is rapidly demethylated to morphine. The extent of morphine formation was very different between rats, causing large SD for the AUC ratio of morphine to codeine. In comparison to codeine, morphine has a 10-times lower lipid/water partition coefficient (1), which result in lower brain uptake. In accordance, no morphine was detected in brain MD, while total

concentrations were detected, although with a very large variability.

In conclusion, microdialysis is a useful technique for investigations of BBB transport. Codeine was rapidly transported into the brain and quickly reached distribution equilibrium with the same unbound concentrations in blood and brain. The influx and efflux clearances across the BBB were thus equal.

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