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A microdialysis model to examine nasal drug delivery and olfactory absorption in rats using lidocaine hydrochloride as a model drug

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Abstract

Targeting of the central nervous system by direct drug transport from the nose to the brain has gained increased attention through the last decade. In the present study, a model for olfactory drug absorption has been investigated using intravenous and unilateral nasal administration of lidocaine hydrochloride in rats. To investigate the possible drug delivery aspects of this route of transport to a central part of the brain a microdialysis model using in vivo recovery by calibrator was applied to the systemic blood and to right and left striatum. The integrity of the blood–brain barrier was evaluated following microdialysis probe implantation. The in vivo experiments were carried out as a cross-over study in rats. The drainage from the nasal cavity was not restricted by occlusion. It was found that true unbound lidocaine concentrations could be calculated from in vivo recovery measurements of retrodialysis of prilocaine hydrochloride. The relative in vivo recoveries in striatum (11.3%) and blood (24.0%) were significantly lower than in vitro (31.3 and 44.9%). The blood–brain barrier was found to retain its physical integrity when evaluated one hour after probe implantation. From pharmacokinetic modelling of the time–concentration curves it was found that the absorption rates and area under the curve (AUC) values of lidocaine in left and right striatum were not statistically different following nasal and intravenous administration, respectively. The average nasal bioavailabilities of lidocaine in blood, left and right striatum were 85, 103 and 129%, respectively. It was concluded that no significant olfactory absorption to striatum was evident in the present study. However, the method should be applicable to studies of drug delivery to blood and brain following nasal administration of other drugs.

Keywords: Olfactory pathway; Nasal drug delivery; Microdialysis; Pharmacokinetics; Blood–brain barrier integrity; Lidocaine; Rats

1. Introduction

Delivery of drugs to the central nervous system (CNS) is often compromised by limited drug permeability from the blood across the blood–brain barrier (BBB) or the blood–cerebrospinal fluid (CSF) barrier, which are formed by tight junctions connecting the cerebral endothelial and epithelial cells of the choroid plexi, respectively. Lipid soluble substances with a molecular weight (MW) less than 600 Da may readily permeate the BBB depending on their partitioning coefficients (Levin, 1980; Pardridge, 1991). Lipid soluble molecules with MW > 600 Da are normally not transported passively across the BBB in pharmacologically relevant amounts. For lipid insoluble or charged molecules the molecular weight limit is much lower.

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As a potential way of delivering drugs to the CNS by circumventing the BBB olfactory drug absorption, i.e. direct transport of drugs or model substances from the nasal epithelium to the CNS, has been studied by a number of different research groups during the last decades. Souza-Silva et al. (1997) found that dopamine levels, monitored by microdialysis, in the ipsilateral neostriatum were rapidly and significantly increased following unilateral nasal administration of cocaine and amphetamine. The authors concluded that the observations were due to olfactory drug absorption. Dahlin et al. (2000, 2001) have shown significantly increased levels of radioactivity in the CNS following unilateral administration of [^{3}H]-dopamine compared to equal intravenous doses. In mice, radioactivity was primarily located to the ipsilateral bulb, whereas in rats, radioactivity was also significantly higher in the contralateral bulb and other parts of the CNS. A series of studies by Sakane et al. (1991a, 1994, 1995) have shown relationship between transport from the nasal cavity to the cerebrospinal fluid and lipophilicity, dissociation properties and molecular weight, respectively. The same group of authors also found that nasal administration of 0.8 mg of the water-soluble antibiotic drug cephalexin to rats yielded a 160-fold higher cerebrospinal drug concentration 15 min after administration compared with duodenal (0.2 mg) and intravenous administration (0.2 mg). The difference was approximately 100-fold after 30 min (Sakane et al., 1991b).

The olfactory transport pathway has been reviewed by Mathison et al. (1998) and Illum (2000). Both reviews summarise the hypothesised olfactory transport pathways, methods for studying drug transport from the nasal cavity to the CNS and the findings of various research groups.

A microdialysis model has previously been used by Chou and Donovan (1998a) to study the disposition of lidocaine in the CNS following unilateral nasal or intravenous administration to rats. The animals used in the study were anaesthetised and prepared according to the method described by Hussain et al. (1980) in which the trachea and oesophagus are cannulated and the nasopalantine duct closed, leading to occlusion of the nasal cavity. Microdialysis probes were placed in the cerebrospinal fluid in cisterna magnum, in cerebellum and in left and right olfactory bulbs. A dosage volume of 50 μl lidocaine solution was administered to the right nostril of the animals receiving the nasal formulation. Unbound extracellular concentrations of lidocaine were calculated by calibrating the measured dialysate concentrations with respect to in vitro recovery i.e. the fraction of lidocaine extracted from an aqueous solution by the dialysis probe. Lidocaine concentrations in the right ipsilateral olfactory bulb during the initial 20 min after unilateral nasal administration were significantly higher than in the left, contralateral olfactory bulb. Further, the $AUC_{0–300\,min}$ in sampled CSF was 54% higher following nasal administration compared with equal doses administered intraarterially (Chou and Donovan, 1998b). This difference, however, was not found when lidocaine was sampled by microdialysis.

Microdialysis is a technique which can be used for sampling of drugs from extracellular fluid (ECF) without removal of liquid. The technique enables simultaneous sampling of unbound and therefore pharmacologically active drug fractions as a function of time from various tissues. The principle of microdialysis is diffusion across a semi-permeable membrane in a hollow dialysis fibre. The fibre with a typical cut-off value of 6–20 kDa is perfused with a salt solution that resembles the extracellular fluid of the tissue in which the probe is implanted.

When investigating brain pharmacokinetics by microdialysis it is important to ensure that the BBB is not damaged by probe implantation, in particular when a drug with limited access to the CNS is studied. Tossman and Ungerstedt (1986) have previously described the use of radioactively labelled sodium technetate ($Na^{99m}TcO_4$) as a marker for blood–brain barrier integrity. Sodium technetate, which is impermeable to intact brain capillaries, was intravenously administered to rats together with ^3H_2O 80 min after microdialysis probe implantation in the brain. After measuring the brain dialysate/plasma concentration ratios of the two substances it was concluded that the blood–brain barrier was intact. Other studies of blood–brain barrier integrity shortly after probe implantation, which is essential when working with anaesthetised animals, have indicated that microdialysis studies can be performed on the day of implantation. These studies include Benveniste et al. (1984), Terasaki et al. (1992) and Dykstra et al. (1992) who

used ^{14}C-α-aminoisobutyrate (quantitative autoradiography), ^{14}C-sucrose (brain/plasma concentration ratios) and Evans Blue (visual inspection), respectively, as integrity markers. Sodium fluorescein has previously been used as a marker of BBB integrity by de Lange et al. (1998), who found that the permeability of fluorescein was low and unchanged in mdr1a (−/−) mice compared to the wild-type. Fluorescein has a molecular weight of 330 Da, is negatively charged at physiological pH and can be quantified in very low concentrations in biological media (Selan et al., 1985). This makes fluorescein a suitable substance for evaluating physical blood–brain barrier integrity.

The aim of the present study was to apply a microdialysis model using in vivo recovery determinations for investigating the disposition of lidocaine in blood and brain in rats following a single unilateral nasal administration or an intravenous bolus injection in a cross-over design. To evaluate the potential drug delivery aspects of olfactory absorption to a central part of the brain, microdialysis samples were collected from left and right striatum which is the site of action for many drugs. The nasal cavities of the animals were un-modified in order to promote normal mucociliary clearance of the nasal formulation from the epithelium and to avoid possible tissue damage due to prolonged contact between the mucosa and the formulation. The results of the study should be compared to those obtained by Chou and Donovan (1998a,b). The microdialysis method was validated regarding blood-barrier integrity following probe implantation and simultaneous sampling of arterial blood was used as a control parameter for the in vivo recovery determinations. The presented microdialysis study should serve as a model for further investigations of compounds with low BBB permeability.

2. Materials and methods

2.1. Chemicals

Lidocaine hydrochloride and prilocaine hydrochloride was purchased from Sigma Chemicals (St. Louis, USA). Sodium fluorescein was from ICN Biomedicals Inc. (Ohio, USA). All salts and solvents used were of analytical grade.

2.2. Microdialysis equipment

Microdialysis probes (CMA12/4 and CMA20/10), pumps (CMA/100), in vitro stand (CMA/130) heating plate (CMA/150) glass syringes, drills, stainless steel screws, tubing (i.d. 0.4 mm) and tube fittings were obtained from CMA/Microdialysis AB (Solna, Sweden). The stereotaxic frame was from David Kopf Instruments (Tujunga, USA). Dental Cement was from AgnThos (Stockhom, Sweden).

2.3. Formulations and artificial CSF

A lidocaine solution of 600 mg/ml in sterile water was prepared for nasal administration. This solution was diluted 1 + 19 with sterile saline to a concentration of 30 mg/ml for intravenous injection. Aliquots of both formulations were kept at 4 °C until the day of the experiment. The formulations were chemically and physically stable within the study period.

Ringer's solution (145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.1 mM ascorbic acid, 2.0 mM KH_2PO_4 and 2.0 mM K_2HPO_4 adjusted to pH 7.4) was used as perfusion fluid in microdialysis probes, both in blood and brain.

2.4. In vitro recovery validation

The in vitro recoveries of the probes, i.e. the fraction of lidocaine and prilocaine extracted from an aqueous solution (recovery by gain) or the fraction lost from the perfusate to blank dialysis solution (recovery by loss), for use in brain (CMA-12) and blood (CMA-20) were tested prior to the animal experiments. Three probes of each type were tested with four different concentrations of lidocaine and prilocaine hydrochloride in Ringer's solution (1, 5, 10 and 15 μg/ml). Both recovery by gain and loss (retrodialysis) was investigated at flowrate 2.0 μl/min. In the determinations of recovery by gain, the probes were placed in centrifuge tubes and 1500 μl Ringer's solution containing the drugs was added and replaced every hour of the experiment. At the first 60 min of the experiment, blank Ringer's solution was added to the centrifuge tubes and every 60 min the concentration of lidocaine and prilocaine hydrochloride was increased by replacing the drug solution in the tubes. The perfusate was blank Ringer's solution throughout

the experiment. Determinations of recovery by loss were performed in the same way, except that concentrations in the perfusate were changed accordingly and that the blank Ringer's solution in the centrifuge tubes was replaced every hour to maintain sink condition. Recoveries were calculated as:

$$\text{Recovery} = \frac{(C_{\text{dialysate}} - C_{\text{perfusate}})}{(C_{\text{medium}} - C_{\text{perfusate}})},$$

where $C_{\text{dialysate}}$ was the concentration of drug in the collected outflow from the probes, $C_{\text{perfusate}}$ the drug concentration perfused into the probes and C_{medium} the drug concentration in the centrifuge tubes. The C_{medium} was considered to remain unchanged between replacements. Samples of 20 μl were collected over 10-min intervals and three of the samples from each period were analysed (first two and last sample discarded). The resulting average recoveries from each sampling period from each probe were used in the further calculations. The recovery experiments (gain and loss) were performed on two types of probes ($n = 3$) and four concentration levels. Statistical analysis (ANOVA) of the recovery of each type of probe, concentration level etc. was performed by Statistica 6.0 from Statsoft Inc. (Tusla, USA).

2.5. Animal preparations

The study was performed on male Sprague–Dawley rats ($n = 5$), weighing approximately 350 g, with free access to food and water. The animals were anaesthetised by a subcutaneous injection of a Hypnorm/Dormcium/water mixture (1:1:2) and placed on a heating plate to maintain a normal body temperature (37.5 °C). The arteria carotis was cannulated by a heparin-filled polyethylene catheter for blood sampling. A microdialysis probe (CMA-20) was inserted into the left vena jugularis by a guide cannula and the head of the probe was fixed to the breast musculature. The animal was then placed in the stereotaxic frame and the scull was exposed by an incision in the scalp. A stainless steel screw was placed in a 0.5 mm hole drilled in the scull. Two 1 mm holes were carefully drilled through the scull by a trephine drill at 0.8 mm anterior and ±2.7 mm laterally relative to the Bregma. The Dura was perforated by a needle and microdialysis guides fitted with dummies were implanted through the holes and fixed to the scull with dental cement, secured by the stainless steel screw. After 30 min the cement was hardened, the dummies were removed and the probes (CMA-12) inserted through the guides. The ventral position of the tip of the probe was 4.7 mm relative to Bregma, leaving the entire membrane surface of the brain probes in left and right striatum, respectively. The experimental animals were kept under anaesthesia during the entire study (7–8 h) and were killed by arterial bleeding at the end of the experiment.

2.6. Blood–brain barrier integrity assay

The integrity of the blood–brain barrier 1 h after probe implantation was evaluated prior to each experiment. This was done by intravenous injection of 1 mg sodium fluorescein and subsequent sampling for 60 min at 10-min intervals from the probes in both brain and blood. Analysis of sodium fluorescein concentrations in the samples was performed by HPLC with fluorescence detection (Merck L-7480). The excitation wavelength was 495 nm and emission was measured at 510 nm. $AUC_{0–60\,\text{min}}$ was calculated for each probe by the trapezoid method and the $AUC_{\text{brain}}/AUC_{\text{blood}}$ ratios were used as a measure of integrity.

2.7. Pharmacokinetic studies

Following the integrity assay, intravenous or unilateral nasal administration was given. The intravenous formulation (200 μl) was injected in the tail vein. Nasal administration was given 15 mm into the cavity as a droplet on the mucosa in the right nostril by inserting a soft catheter fitted to a 25 μl Hamilton microsyringe. The dosage volume was 10 μl. The rats were kept on the abdominal side throughout the study to retain as much mucociliary activity of the nasal epithelium as possible despite the use of anaesthesia. A cross-over design was used for the studies. In three of five animals, lidocaine was first administered nasally and later, after a washout period and blank sampling, the drug was intravenously injected. Two animals received the intravenous administration first. Fractions of dialysate from the microdialysis probes were sampled with 10-min intervals for 120 min after each administration. Before administering lidocaine to the

experimental animals, blank samples were collected. Blank samples were also collected at the end of each washout period to ensure lidocaine concentrations below the limit of detection prior to the following administration. A perfusion fluid containing 3.0 μg/ml of prilocaine hydrochloride, a drug resembling lidocaine hydrochloride in its physicochemical properties, was used to assess the in vivo recovery throughout the studies at a flow-rate of 2.0 μl/min. The in vivo recovery of prilocaine was used for calculating true unbound concentrations of lidocaine. This was done by measuring the average recovery by loss of prilocaine from each probe. Calculations are shown in Section 2.9. Blood samples of 200 μl were taken from the arterial catheter at 0, 30, 60, 90 and 120 min. The blood was sampled in heparin-coated vials and centrifuged at 1700 G for 10 min to isolate the plasma, which was stored at −20 °C until analysis.

2.8. Analysis

Concentrations of lidocaine and prilocaine were determined by HPLC-UV analysis. Lidocaine standards were prepared from the intravenous formulation and diluted with Ringer's solution to relevant concentrations. Standards of prilocaine were also prepared in Ringer's solution. The HPLC system (all from Merck, Darmstadt, Germany) consisted of a pump (L-6000) with flow-rate 1 ml/min, detector (L-4000) with a detection wavelength of 210 nm, column (LiChrospher 100 RP-18 5 μm) and an integrator (D-7500). All peak analysis and integration was performed by Merck HPLC System Manager 3.0. The retention time of prilocaine and lidocaine was 6 and 10 min, respectively. The limit of detection of lidocaine and prilocaine was 0.15 and 0.10 μg/ml, respectively, using 15 μl sample volumes. The linear range of the analytical method was 0.3–30 μg/ml for both lidocaine ($r^2 = 0.995$) and prilocaine hydrochloride ($r^2 = 0.998$) and the coefficient of variance (CV) of was 5.4% at 0.3 μg/ml and 2.8% at 30 μg/ml. For plasma samples, an on-line extraction system as described by Bechgaard et al. (1997) was used for purification. All plasma samples and standards were diluted five times prior to injection onto the HPLC system. The lidocaine extraction recovery from plasma was approximately 85% compared to directly injected aqueous standards.

2.9. Calculations

In vivo recovery of prilocaine was calculated from the following equation

$$\text{Recovery(prilocaine)} = \frac{(\text{Cpri}_{\text{dialysate}} - \text{Cpri}_{\text{perfusate}})}{(\text{Cpri}_{\text{ECF}} - \text{Cpri}_{\text{perfusate}})},$$

where Cpri (prilocaine concentration) is given for dialysate, perfusate extracellular fluid (brain interstitial fluid or plasma). Due to the high diffusion coefficient in biological tissues of prilocaine, Cpri_{ECF} was assumed to equal zero during the retrodialysis period. Since in vivo retrodialysis recoveries of lidocaine and prilocaine have previously been found to be equal in the brain at different concentrations (pilot study, unpublished results), recoveries of lidocaine were calculated as:

$$\text{Recovery(lidocaine)} = \left(1 - \frac{\text{Cpri}_{\text{dialysate}}}{\text{Cpri}_{\text{perfusate}}}\right)$$

Unbound extracellular concentrations were then calculated as:

$$C_{\text{ECF}}\ \text{(lidocaine)} = \frac{\text{Clid}_{\text{dialysate}}}{(1 - \text{Cpri}_{\text{dialysate}}/\text{Cpri}_{\text{perfusate}})},$$

where Clid is the lidocaine concentration. The calculated extracellular unbound concentrations of lidocaine following nasal or intravenous administration were used in pharmacokinetic modelling by WinNonlin 2.1. For each time–concentration profile, a one-compartment model with first order elimination was fitted to the data. The model with first order absorption was used to describe the pharmacokinetics in striatum (after both nasal and intravenous administration) as well as in plasma following nasal administration. Various pharmacokinetic parameters were derived from the fitted curves of unbound concentrations in striatum and blood as well as from total plasma. The free fraction of lidocaine in blood was calculated as $\text{AUC}_{0\text{–}120\,\text{min}}$ (ECF)/$\text{AUC}_{0\text{–}120\,\text{min}}$ (plasma) following intravenous administration.

3. Results

3.1. Recovery

The in vitro experiments showed no difference between the individual probes or concentrations used in the test. As seen from Table 1, recoveries from the blood probes were much higher than from the brain probes which was expected due to the difference in membrane lengths (10 and 4 mm, respectively). The statistical analysis showed no difference ($P > 0.05$) between the four drug concentration levels tested. Also no difference ($P > 0.05$) was found between the three individual probes of each type used in the experiment. For both CMA-12 and CMA-20, a significant difference ($P = 0.033$ and $P = 0.003$, respectively) was found between recoveries obtained by gain and loss. For CMA-20, no significant difference between lidocaine and prilocaine hydrochloride was found, whereas a difference ($P = 0.035$) was found with CMA-12. The differences, however significant, were quite small. From Table 1 it is also seen that average in vivo recovery value from the brain probes was 11.3% (S.D. 1.1%), whereas recovery in blood was 24.0% (S.D. 1.8%). The recoveries of the left and right brain probes were 10.8% (S.D. 1.2%) and 11.9% (S.D. 0.8%), respectively. Despite the minor differences between the methods of in vitro recovery determination of the two drugs, lidocaine concentrations in all microdialysis samples were corrected by the average in vivo retrodialysis recovery of prilocaine of the corresponding probe.

3.2. Blood–brain barrier integrity

To evaluate the blood–brain barrier integrity the area under the curve (AUC) of sodium fluorescein was calculated and the brain/blood ratios of fluorescein $AUC_{0–60}$ min were found for each experiment. The resulting average ratios were 2.8% (S.D. 0.7%) and 2.7% (S.D. 0.8%) in left and right striatum, respectively, indicating that the physical integrity of the blood–brain barrier was retained. This conclusion is based on the assumption that the ratio between the in vivo recoveries of fluorescein in brain and blood is approximately the same as seen with prilocaine and that a disruption of blood vessels during probe implantation would lead to high $AUC_{0–60\,min}$ ratios between brain and blood. The presence of fluorescein in the samples did not interfere with the quantification of lidocaine and prilocaine in microdialysis and plasma samples.

3.3. Pharmacokinetic study

The calculated unbound concentrations of lidocaine in left and right striatum and in blood are shown in Fig. 1. It is seen that almost identical time–concentration profiles in brain and blood were found following nasal administration (a), whereas concentrations were higher in blood compared to the brain in the first 20 min after intravenous injection as expected (b). Selected pharmacokinetic parameters calculated from the individual time–concentration profiles from the microdialysis experiments are shown in Table 2, where values of AUC, C_{max}, absorption

Table 1
Average recoveries in vitro ($n = 12$; three probes, four concentraion levels) and in vivo ($n = 5$) of prilocaine and lidocaine hydrochloride from probes used in brain (CMA-12) and blood (CMA-20)

	Recovery (%)			
	Lidocaine, HCl		Prilocaine, HCl	
	Gain	Loss	Gain	Loss
CMA-12[a] in vitro	28.2 (2.0)	30.9 (2.1)	29.7 (2.0)	31.3 (2.3)
CMA-12[a] in vivo				11.3 (1.1)
CMA-20[b] in vitro	42.5 (3.0)	46.3 (2.8)	41.9 (3.1)	44.9 (2.4)
CMA-20[b] in vivo				24.0 (1.8)

In vitro recoveries were tested at four different concentration levels (1, 5, 10 and 15 μg/ml). A perfusate flowrate of 2.0 μl/min was used in all experiments. Numbers in parentheses indicate standard deviations.

[a] Probes for use in brain.

[b] Probes for use in blood.

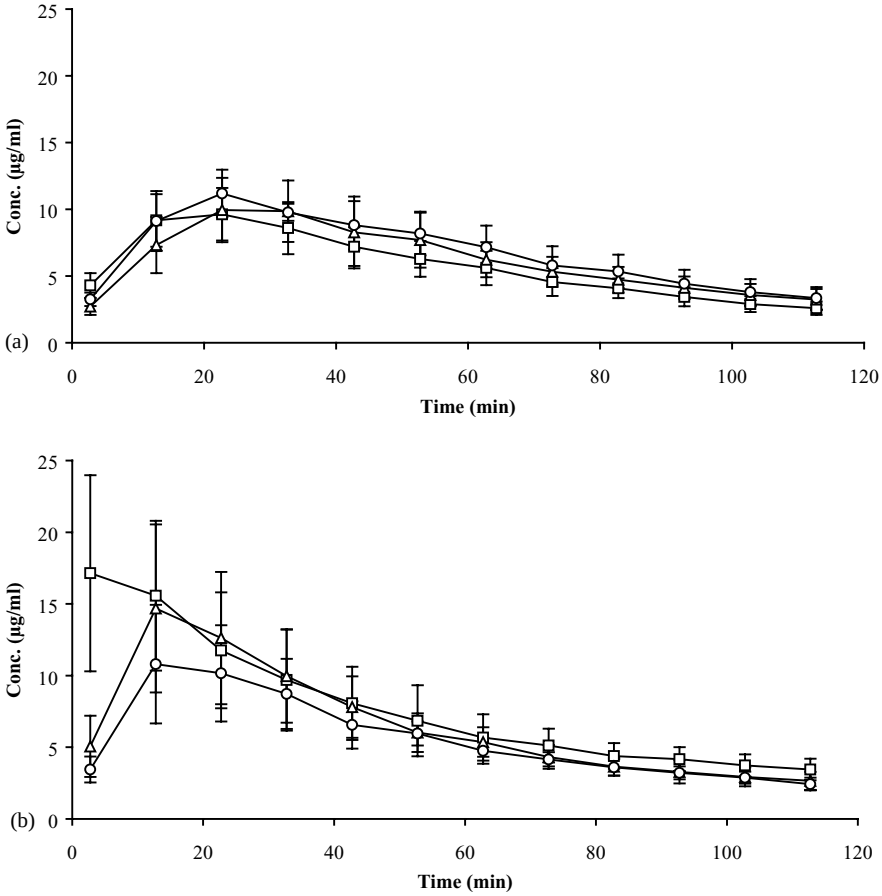

Fig. 1. Unbound concentrations following (a) intranasal and (b) intravenous administration of 6 mg lidocaine hydrochloride to rats ($n = 5$). Squares, triangles and circles represent lidocaine concentrations sampled by microdialysis in blood, left striatum and right striatum, respectively. All data are corrected by in vivo recoveries. Bars represent standard deviations.

Table 2
Pharmacokinetic parameters of nasally or intravenously administered lidocaine hydrochloride to rats ($n = 5$)

Parameter	Left striatum		Right striatum		Blood	
	i.n.	i.v.	i.n.	i.v.	i.n.	i.v.
AUC (min µg ml^{-1})	875 (184)	855 (198)	978 (213)	774 (133)	837 (165)	1064 (283)
AUC (min µg ml^{-1})[a]						4297 (737)
C_{max} (µg ml^{-1})	9.6 (2.4)	13.6 (5.2)	10.7 (1.5)	10.4 (3.4)	9.7 (2.0)	18.1 (7.4)
K01 ($\times 10^{-3}$ min^{-1})	71.8 (28.9)	160 (39.4)	87.7 (39.8)	148 (52.6)	148 (19.8)	
K10 ($\times 10^{-3}$ min^{-1})	18.1 (3.1)	21.6 (5.8)	18.4 (5.7)	18.4 (5.3)	15.2 (1.9)	16.7 (3.3)
K10 ($\times 10^{-3}$ min^{-1})[a]					15.5 (1.9)	15.1 (3.4)
T_{max} (min)	26.9 (4.8)	14.9 (2.2)	24.5 (6.1)	16.9 (3.4)	17.3 (1.5)	

Samples were collected by microdialysis in blood and in right and left striatum. Average values of $AUC_{0–120\,min}$, C_{max}, absorption rate, half-life and T_{max} were calculated by WinNonlin from individual time–concentration profiles. Numbers in parentheses indicate standard deviations.

[a] Values obtained from total plasma data.

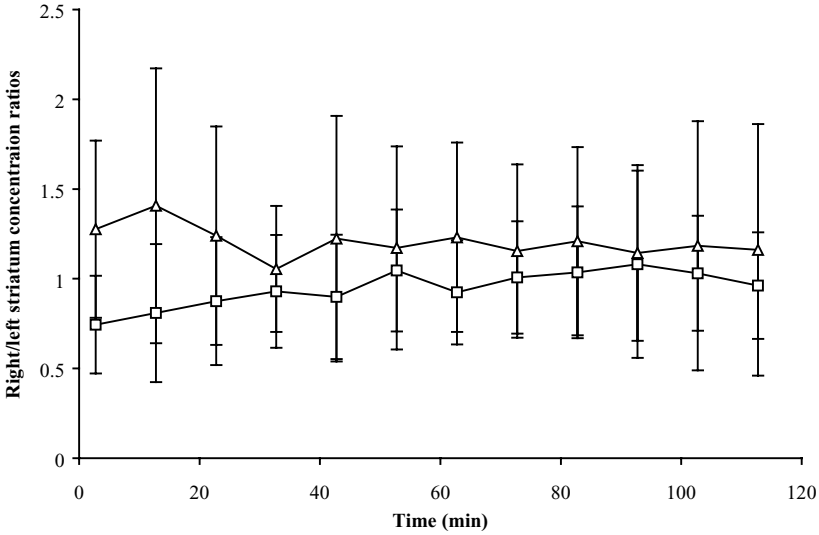

Fig. 2. Average unbound concentration ratios between right and left striatum following nasal (triangles) and intravenous administration (squares) as a function of time. Bars indicate standard deviations ($n = 5$).

rate (K01), half-life (K10) and T_{max} can be found. The correlations of individual fittings (r^2) between observed and predicted values of unbound lidocaine concentrations were in the range of 0.940–0.998. The average absorption rate of lidocaine to the brain was 0.080 and $0.154\,min^{-1}$ following intravenous and nasal administration, respectively. No significant differences between left and right striatum were found (t-test) for any of the calculated pharmacokinetic parameters. The level of significance between left and right striatum in absorption rates were $P = 0.42$ and $P = 0.23$ for nasal and intravenous administration, respectively. For AUC the levels were $P = 0.59$ and $P = 0.47$ accordingly. In addition, the average AUC-value in blood following intravenous administration and the average half-life following both

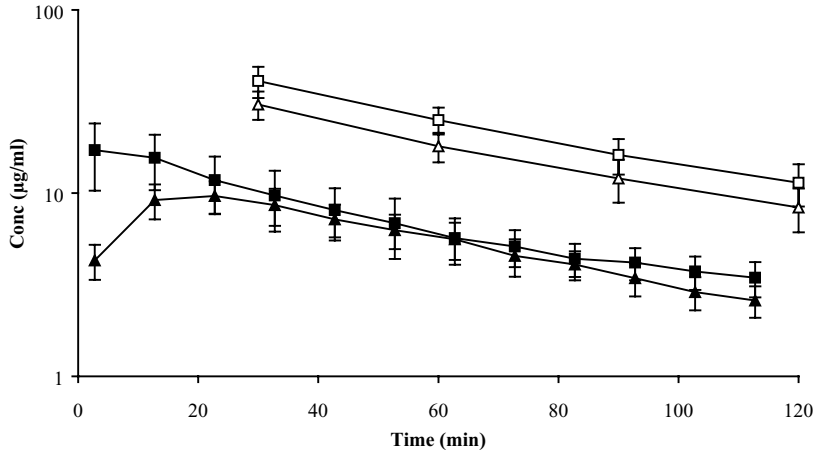

Fig. 3. Plasma concentrations of lidocaine following nasal (triangles) and intravenous (squares) administration ($n = 5$). Open symbols indicate average total plasma concentrations obtained from direct blood sampling. Closed symbols indicate average unbound (in vivo recovery corrected) concentrations obtained by microdialysis in blood. Bars represent standard deviations.

intravenous and nasal administration was calculated from total plasma concentrations and added to the table for comparison.

For each microdialysis experiment, samples collected prior the lidocaine administration and at the end of each wash-out period were below the limit of detection (1.5 μg/ml) with respect to lidocaine. From the individual AUC-values the bioavailability of nasally administered lidocaine hydrochloride was calculated ($AUC_{i.n.}/AUC_{i.v.}$) in blood (85%) and in both left and right striatum (103 and 129%). No statistical difference between the lidocaine bioavailabilities in left and right striatum was found ($P = 0.16$). For each microdialysis sampling interval, ratios between right and left striatum were calculated. The average ratios following nasal and intravenous administration are shown in Fig. 2 as a function of time. It is seen that the ratios after both routes of administration are close to unity.

The average plasma concentrations of lidocaine from blood samples taken during the microdialysis experiments are shown in Fig. 3 together with unbound concentrations of lidocaine sampled by microdialysis. The blood samples were taken to enable validation of the in vivo recovery determination by comparing the ratio between calculated free fractions and total blood levels of lidocaine to literature values of plasma protein binding. For this reason only a limit number of samples were taken. It is seen that plasma concentrations are approximately four times higher than the calculated unbound concentrations and that the elimination rates in plasma and dialysates are parallel.

4. Discussion

As an evaluation of the microdialysis method for studies of olfactory drug delivery, it has been shown that it is possible to estimate relevant pharmacokinetic parameters of lidocaine in brain and blood following nasal and intravenous administration. Even though it would be possible to make some relative comparisons between i.e. right and left side of the brain without knowledge of the true, unbound drug concentrations, the use of in vivo recoveries enables verification of the results. Commonly used is the method of retrodialysis by calibrator as described by Stahle et al. (1991); Bouw and Hammarlund-Udenaes (1998) where a reference substance with comparable physio-chemical properties is added to the perfusion fluid and recovery is determined by measuring the loss as the concentration difference between perfusate and dialysate relative to the perfusate concentration. By use of a calibrator substance it is possible to measure recovery throughout the actual study. The diffusion characteristics in e.g. brain tissue of the calibrator substance should resemble those of the drug as closely as possible to obtain valid recovery measurements. For this purpose, prilocaine hydrochloride has previously been used in studies of cutaneous drug delivery as a calibrator substance for lidocaine (Kreilgaard, 2001) where in vitro and in vivo recoveries of the two substances, using linear microdialysis probes, were not discernible. In the present study, two different types of concentrical probes were used for implantation in brain and blood. Due to the difference in membrane lengths (4 and 10 mm, respectively), the in vitro recoveries from the blood probes were approximately 45% higher than from the brain probes. In vivo, however, this difference was 100%, probably on account of the different environments of implantation. This emphasizes the need for valid in vivo recovery determinations if quantification of true extracellular concentrations is of importance. In the present study, the expected equal unbound lidocaine concentrations in brain and blood were found only by correction by in vivo recoveries (on average 11.3% in brain and 24.0% in blood). This indicates that the in vivo recoveries used for correction are valid and that prilocaine hydrochloride can serve as a calibrator substance for the determination of lidocaine recovery in blood and brain. The validity of the recovery values is further supported by the pharmacokinetic parameters calculated from the plasma data. The average total plasma AUC of lidocaine was approximately four times higher than the AUC of unbound lidocaine, which is in agreement with previous studies by Bruguerolle et al. (1983) who found that the protein binding of lidocaine in rats was approximately 70% with some circadian variation. The calculated free fraction of lidocaine in the present study was 25% (S.D. 3.6%). From Table 2 it is also seen, that the calculated half-lives of lidocaine are similar, regardless of sampling site and method.

As previously mentioned, there is a risk that implantation of microdialysis probes in the brain may affect the physical blood–brain barrier integrity. In the present study an average of less than 3% of

fluorescein was sampled from the brain probes compared to the blood probes. However, these results are based on sampled concentrations since in vivo recovery was not measured. Most likely the in vivo recovery of fluorescein would be higher from blood than from brain probes as seen with prilocaine hydrochloride in the present study. This would result in somewhat larger brain/blood ratios of fluorescein. However, even though integrity was measured only 60 min after probe implantation in this study, similar AUC ratios have been found 24 and 48 h after surgery (unpublished results). In addition, incidental damage to the blood–brain barrier would most likely result in larger variations between experiments than observed (R.S.D. approximately 30%). Sodium fluorescein has previously been used by de Lange et al. (1998) to evaluate the blood–brain barrier integrity in MDR1A (−/−) and wild-type mice. After 2 h of infusion (50 nmol/min), the fluorescein concentration ratio between total brain and plasma was found to be 2.3%. These findings, together with the results of the present study, indicate that fluorescein is a suitable marker of blood–brain barrier integrity.

Pharmacokinetic modelling of unbound concentrations is a valuable tool in studies of olfactory absorption as absorption rates to the brain and other relevant pharmacokinetic parameters can be determined. However, this may be difficult for drugs that are quickly distributed across the blood–brain barrier as microdialysis studies of drug absorption often become a compromise between the number of time-points needed to calculate the absorption rate and the analytical sensitivity available. This means that results from studies of olfactory drug absorption should be easier to interpret if the model substance is poorly absorbed to the systemic blood or having restricted access to the CNS from the blood. The present study shows that lidocaine is easily absorbed across the nasal epithelium and readily permeates the BBB and therefore serves well as a model substance which can be quantified all relevant tissues. The results show that it should be possible to apply the presented microdialysis model, which includes in vivo recovery determination, blood–brain barrier integrity assessment and measurement in a pharmacologically relevant part of the brain, to studies of other drugs or reference substances with restricted access to the CNS. In the present study the nasal bioavailabilities ($AUC_{i.n.}/AUC_{i.v.}$ ratios) in left and right striatum (103 and 129%) were found to be slightly, however insignificantly, higher than in blood (85%). If nasally administered lidocaine should have entered the striatum only via the systemic blood, bioavailabilities would not be expected to exceed 85%. The remaining contribution may originate from olfactory absorption as indicated by be higher bioavailability in the right, ipsilateral side. Although interesting, this possible contribution is small for lidocaine. Even though most CNS-active drugs exert their pharmacological effect in the brain, most studies of olfactory absorption of drugs have been performed using CSF sampling. It was shown by Chou and Donovan (1998a,b) that the CSF concentrations of lidocaine resembled those in the brain, but in general, drug concentrations in the CSF are not indicative of brain extracellular concentrations (Pardridge, 1991). Pardridge also states that the barrier between the subarachnoid space, in which the CSF flows, and the brain parenchyma is functional rather than anatomical and exists due to bulk flow and clearance of the CSF.

In contrast to Chou and Donovan (1998a), who implanted microdialysis probes in the olfactory bulbs, the brain microdialysis samples in the present study were collected from striatum. If olfactory absorption should be used clinically, drug transport to parts of the brain distal to the olfactory bulbs must be achieved. Since striatum is the pharmacological site of action of many drugs this part of the brain was used to evaluate the potential drug delivery to the brain. In addition, implantation of microdialysis probes in striatum is probably less prone to errors due to the size and location of the olfactory bulbs. Chou and Donovan (1998a) found that olfactory absorption, in addition to the systemic circulation, contributes, though of lower magnitude, to the transport to the olfactory bulbs following nasal administration. This was not found in striatum in the present study as differences in absorption rates and bioavailabilities were not significantly different between left and right striatum. However, with the site of implantation approximately 6 mm from the olfactory bulb, the studies are not contradictory. They merely support the theory that hydrophilic compounds that may be following the nose to brain pathway will primarily be found in CSF and the olfactory bulb, as discussed by Illum (2000) who also stated that this route of transport may only be significant for drugs that are poorly absorbed from the

nasal epithelium or have limited blood–brain barrier permeabilities.

As previously mentioned, the nasal cavities of the animals used in the present study were unmodified to prevent excessive absorption and damage to the epithelium. With lidocaine hydrochloride it has been shown by Donovan and Zhou (1995) that the drug itself decreases the ciliary beat frequency in excised nasal mucosa. However, the results obtained in these in vitro systems may overestimate the effects on epithelial clearance observed in vivo. The results of the present study show that it is possible to avoid modification of the nasal cavity without introducing large variations in the absorption data.

In conclusion, it has been shown that relevant pharmacokinetic parameters can be obtained by using microdialysis in studies of olfactory drug absorption. The blood–brain barrier integrity following microdialysis probe implantation was found to be acceptable and measurements of in vivo recovery by retrodialysis of prilocaine hydrochloride seem to provide true unbound concentrations of lidocaine. The validity of the results obtained by microdialysis is supported by the plasma data. From the calculated pharmacokinetic parameters it was further concluded that no significant olfactory absorption to striatum was found. The presented model should be applicable to further studies, including poorly absorbed compounds, of nasal drug delivery to the central brain.

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