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Application of intracerebral microdialysis to study regional distribution kinetics of drugs in rat brain

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1 The purpose of the present study was to determine whether intracerebral microdialysis can be used for the assessment of local differences in drug concentrations within the brain.

2 Two transversal microdialysis probes were implanted in parallel into the frontal cortex of male Wistar rats, and used as a local infusion and detection device respectively. Within one rat, three different concentrations of atenolol or acetaminophen were infused in randomized order. By means of the detection probe, concentration-time profiles of the drug in the brain were measured at interprobe distances between 1 and 2 mm.

3 Drug concentrations were found to be dependent on the drug as well as on the interprobe distance. It was found that the outflow concentration from the detection probe decreased with increasing lateral spacing between the probes and this decay was much steeper for acetaminophen than for atenolol. A model was developed which allows estimation of k_{bp}/D_{eff} (transfer coefficient from brain to blood/effective diffusion coefficient in brain extracellular fluid), which was considerably larger for the more lipohilic drug, acetaminophen. In addition, *in vivo* recovery values for both drugs were determined.

4 The results show that intracerebral microdialysis is able to detect local differences in drug concentrations following infusion into the brain. Furthermore, the potential use of intracerebral microdialysis to obtain pharmacokinetic parameters of drug distribution in brain by means of monitoring local concentrations of drugs in time is demonstrated.

Keywords: Intracerebral microdialysis; brain; distribution kinetics; *in vivo* recovery; atenolol; acetaminophen; blood-brain barrier; steady-state; mathematical model

Introduction

Drug transport across the blood-brain barrier (BBB) is determined by the local morphological and functional characteristics of brain capillaries and the physico-chemical characteristics of the drug. In particular for hydrophilic drugs, the transport into the brain by the paracellular route is restricted because of the presence of tight junctions between capillary endothelial cells in the brain (Bodor & Brewster, 1983; Van Bree *et al.*, 1988; Pardridge, 1988). For several lipophilic drugs the P-glycoprotein, expressed on the luminal face of the cerebral endothelial cells, counteracts the entrance into the brain (Schinkel *et al.*, 1994). Since drug concentrations in target brain areas supposedly determine the intensity of the pharmacodynamic response, knowledge about BBB transport is of interest in particular under those conditions where the local permeability of the BBB may be changed (Banks & Kastin, 1988; Kent & McKendall, 1989; Trnovec *et al.*, 1990).

Intracerebral microdialysis is a relatively new *in vivo* technique by which concentrations of small endogenous compounds in brain ECF can be monitored dynamically (Tossman & Ungerstedt, 1986; Benveniste *et al.*, 1989; Di Chiara *et al.*, 1990). More recently, applications in the field of pharmacokinetics have been reported (Stahle, 1991; Dykstra *et al.*, 1992). An important question is whether intracerebral microdialysis is able to detect local differences in concentrations of drugs within the brain.

Previous microdialysis studies on transport of atenolol and acetaminophen into the brain have already shown that well-defined and reproducible concentration-time profiles in cortical brain dialysate can be obtained after systemic administration (De Lange *et al.*, 1994). However, upon intracerebroventricular (i.c.v.) administration significant interanimal differences in the cortical dialysate concentrations of acetaminophen were observed, while for atenolol the concentrations were below the detection limit of the assay. Upon i.c.v. administration of these drugs, the profiles obtained in cerebrospinal fluid (CSF) after serial CSF sampling resulted in more or less reproducible concentration-time profiles (Van Bree *et al.*, 1989). Therefore, the microdialysis finding may be explained by interanimal differences in 'diffusional distance' from the ventricle to the measuring probe, together with differences in transport across the ependyma layer and CSF clearances of the drugs. These findings suggest that within the brain quite significant concentration differences exist.

Different patterns of spatial distribution of drugs in the brain have been observed in extensive studies on the penetration of various substances into brain tissue. The lipophilicity of the drug under investigation played an important role in this respect. It was demonstrated that following experimental ventriculocisternal perfusion (Fenstermacher *et al.*, 1970; 1974; 1981) and microperfusion (Sendelbeck & Urquhart, 1985), substances that easily cross the cerebral capillaries were transported rapidly into the systemic circulation while substances with low transcapillary transport penetrated more deeply into the brain.

The purpose of the present study was to determine whether intracerebral microdialysis can be used for the assessment of local differences in drug concentrations within the brain. Local infusion (Quan & Blatteis, 1989) of two model drugs with different lipophilicities was expected to result in different patterns of spatial distribution. Two microdialysis probes were implanted in parallel into the brain cortex, and used as an infusion and a detection probe respectively. After local infusion of three different concentrations of acetaminophen or

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atenolol, the concentration-time profiles in cortical brain dialysate of these model drugs were studied at different interprobe distances (1–2 mm), and could be used to determine disposition parameters. The results on the steady-state distribution in brain after infusion were subjected to a mathematical analysis, by which the ratio of the transcapillary transfer coefficient over the apparent diffusion in brain extracellular fluid (ECF) was calculated. In addition, the analysis provided a means of estimating *in vivo* recovery values.

Methods

Animals

Male SPF Wistar rats (180–200 g) were obtained from the Sylvius Laboratory breeding facility and maintained on a commercially available diet (standard laboratory rat diets, RMH-TH, Hope Farms, Woerden, The Netherlands) with free access to water.

Surgical procedure

Rats were anaesthetized with a 0.1 ml kg^{-1} intramuscular injection of Hypnorm and mounted in a stereotactic instrument. Incisions were made to expose the periosteum which was locally anaesthetized with a 0.6% (w/v) solution of lignocaine. Thereafter the periosteum was removed to expose the skull. The mandibular muscle layer was sectioned, exposing the lateral planes of the skull. Two pairs of 1.5 mm holes were drilled in these lateral planes. The use of a tungsten wire allowed for the horizontal (transversal) introduction of the dialysis probes through the cortex by means of a stereotactic manipulator, at 2 mm below the bregma point. The dialysis fibres (o.d. 0.29 mm, C-DAK artificial kidney 201-800 d 135 SCE, CD Medical B.V. Rotterdam, The Netherlands) used in this study had been previously covered with silicone glue (Rhodosil CAF 3, Rhone-Poulenc, Amstelveen, The Netherlands) except for an 8 mm length to be positioned centrally in the cortex. The infusion probe was implanted 0.5 mm behind the bregma while the position of the detection probe was chosen between 0.5 to 1.5 mm before the bregma. The interprobe distance (r) for each individual rat was determined after this implantation procedure (±0.05 mm) at both sides of the skull and averaged. Thereafter, the tungsten wires were gently pulled out of the dialysis membranes. Stainless still needles, glued to both ends of the dialysis fibres, were secured with dental cement on the top of the skull. The animals were allowed to recover from probe implantation and anaesthesia for about 24 h before the start of the experiment.

A perfusate temperature of 38°C was achieved by using two subcutaneous cannulae (polyethylene tubing, i.d. 0.58 mm, length ± 20 cm) on the back of the rat (De Lange *et al.*, 1994). The perfusate fluids were led through these cannulae just before entering the microdialysis probes in order to equilibrate to rat body temperature before entering the microdialysis probe.

Experimental procedure

For the microdialysis experiments with local infusion and detection, the stainless steel needles at one site of the microdialysis probes were connected to a perfusion pump (Gilson Minipulse 2, Villiers le Bel, France) by means of polyethylene tubing (o.d. 0.61 mm, i.d. 0.28 mm). The flow in both probes was in the same direction, with a rate of 7 μl min^{-1}. The detection dialysis probe was perfused with a 2 mM phosphate buffer containing 145 mM sodium, 2.7 mM potassium, 1.2 mM calcium, 150 mM chloride, 1.0 mM magnesium (all as ions), and 0.2 mM ascorbate, pH = 7.4. At the other side the needles were also connected to polyethylene tubing (o.d. 0.61 mm, i.d. 0.28 mm, 80 cm) and the dialysate from the detection probe was collected in the sample loop of an h.p.l.c. system. Concentrations of acetaminophen and atenolol in brain dialysate were determined on-line. The dialysate from the infusion probe was collected in fractions and determined off-line.

At the beginning of the experiment both probes were perfused with buffer solution for 30 min to obtain dialysis equilibrium and blank data. Thereafter, the infusion probe was perfused with solutions of atenolol and acetaminophen in saline at a flow rate of 7 μl min^{-1}. Acetaminophen solutions were pH neutral (≈6–7) while the basic atenolol solutions needed to be adjusted to pH = 7.4 with 0.1 N HCl without changing osmolarity significantly. Three different infusion concentrations were used in randomized fashion: 3.9/5.9/7.8 mM for atenolol ($n = 13$) and 0.16/0.51/1.3 mM for acetaminophen ($n = 14$). The drug delivery rate, reflected by *in vivo* loss of the drug from the infusion probe, was to be determined for each experiment by the off-line analysis of the dialysate; however, after the experiments, it appeared that the off-line measurements of the drugs displayed too much variability to allow a reliable estimation of drug delivery. Each infusion concentration was constantly infused until a (semi)steady-state level was achieved in the dialysate of the detection probe, indicating a more or less constant drug delivery from the infusion probe at that time.

Drugs

Atenolol was purchased from Bufa B.V. (Castricum, The Netherlands), Hypnorm from Janssen Pharmaceutica (Goirle, The Netherlands), acetaminophen and lignocaine from the Pharmacy of Leiden University Hospital (Leiden, The Netherlands).

Drug analysis

Acetaminophen The acetaminophen h.p.l.c. system consisted of a reversed phase column (Spherisorb, 10 cm × 4.6 mm i.d., S3 ODS2, Phase Separations, Waddinxveen, The Netherlands), a precolumn (Pellicular reversed phase, Chrompack, Middelburg, The Netherlands), a Spectraflow 400 solvent delivery system (Kratos Analytical Instruments, SF 400, Rotterdam, The Netherlands) and an electrochemical detector (glassy carbon electrode with an oxidation potential of 800 mV versus a saturated Ag/AgCl electrode, Antec Leiden B.V., Leiden, The Netherlands). The mobile phase consisted of a mixture of a 20 mM sodium phosphate and a 50 mM sodium citrate buffer containing 0.25 mM sodium octane sulphonic acid and 15% (v/v) methanol, pH = 3.0. The flow rate was 1.0 ml min^{-1}. Chromatographic data were recorded and processed with a SP4100 computing integrator (SP4100 computing injector, Spectra Physics B.V., Eindhoven, The Netherlands). The microdialysate was injected on-line into the h.p.l.c. system through an injection valve (Valco Europe, VICI AG) equipped with a 16 μl loop with a repetition time of 4.3 min. The range of concentrations used for calibration (5–500 nM) showed good linearity ($r \geq 0.9998$). The detection limit was 3 nM (50 fmol absolute) and the coefficient of variation in aqueous solution was 1.6% ($n = 12$).

Atenolol The atenolol h.p.l.c. system consisted of a reversed phase column (Spherisorb, 10 cm × 4.6 mm i.d., S3 ODS2, Phase Separations, Waddinxveen, The Netherlands), a precolumn (Pellicular phase, Chrompack, Middelburg, The Netherlands), a Spectraflow 400 solvent delivery system (Kratos Analytical instruments, SF 400, Rotterdam, The Netherlands) and a Shimadzu RF-350 Fluorimeter (Shimadzu Corp., Kyoto, Japan) with an excitation wavelength of 276 and an emission wavelength of 309 nm. The mobile phase consisted of 75% (v/v) sodium acetate buffer pH = 4.0 containing 5 mM sodium octane sulphonate and 25% (v/v) of acetonitrile. The flow rate was 1.0 ml min^{-1}. The microdialysate was injected on-line into the h.p.l.c. system through a Valco injection valve equipped with a 16 μl loop with a repetition time of 3.3 min.

The range of concentrations used for calibration (0.4–15 μM) showed good linearity ($r \geq 0.9992$). The detection limit was 0.4 μM (6 pmol absolute) and the coefficient of variation in aqueous solution was 4% ($n=12$).

Data analysis

In vitro recovery was determined as the concentration of drug in the dialysate in relation to the concentration of the drug in the non-stirred bulk perfusion solution at 37°C (C_{dial}/C_{bulk}). The *in vitro* recovery values (mean ± s.e.mean) for 8 mm of semipermeable dialysis membrane were 18 ± 0.9% and 10 ± 1% for acetaminophen ($n=3$) and atenolol ($n=3$) respectively.

The concentration-time profiles of the drugs as determined in the dialysate from the detection probe ($C_{t,\,dial}$), were divided into three parts according to the three different infusion concentrations (C_{inf}). For each part of $C_{t,\,dial}$, the steady-state concentrations ($C_{ss,\,dial}$) were determined graphically if steady-state conditions were achieved during the infusion as for acetaminophen (Figure 1a), or calculated using the one compartment infusion model:

$$C_{t,\,dial} = C_{ss,\,dial} \times [1 - e^{-kss.t}] \quad (1)$$

(Siphar, SIMED, Creteil, France) for atenolol. The 'end of interval' concentrations obtained after atenolol infusion were >66% of the estimated $C_{ss,\,dial}$ values.

The relationship between the different C_{inf} and the resulting $C_{ss,\,dial}$ appeared to be linear for both drugs (Figure 2), so there was no important concentration-dependency. Therefore, the ratio of $C_{ss,\,dial}/C_{inf}$ (normalized $C_{ss,\,dial}$ values; C) could be used in the subsequent calculations.

Model

The spatial distribution of a drug from the cylinder-shaped microdialysis probe is in the essence two-dimensional. Assuming that: (a) transport of the drug through brain tissue in this experimental design is two-dimensional; (b) no metabolism occurs in brain tissue; and (c) the arterial drug concentrations are small in comparison to the brain ECF concentrations, the relationship between measured drug concentration and distance to site of measurement (Dykstra *et al.*, 1992) as steady-state can be described by:

$$D_{eff} \times \frac{1}{r}\frac{d}{dr} \times \left[r \times \frac{dC}{dr}\right] - k_{bp} \times C = 0 \quad (2)$$

which can be rewritten as

$$D_{eff} \times \frac{1}{r}\left[\frac{dC}{dr} + \frac{d^2C}{dr^2}\right] - k_{bp} \times C = 0 \quad (3)$$

or:

$$\frac{d^2C}{dr^2} + \frac{1}{r} \times \frac{dC}{dr} - \left[\frac{k_{bp}}{D_{eff}}\right] \times C = 0 \quad (4)$$

with the boundary conditions:

$$C(r \rightarrow 0) = C_o \quad (5)$$

$$C(r \rightarrow \infty) = 0 \quad (6)$$

where C is C_{ss}, dial normalized to C_{inf}, k_{bp} is the transcapillary transfer coefficient from brain to plasma, r is the interprobe distance, and D_{eff} is the effective diffusion coefficient through brain ECF.

The model is completely described by the independent parameters C_0 and k_{bp}/D_{eff}. The solution to the second order differential equation is obtained numerically using a fifth order Runge-Kutta method with fourth order step-size control using the computer programme MATLAB and SIMULINK (The Matworks Inc., Natick, Massachussets, U.S.A.). The value of $\frac{dC}{dr}$ at $r \rightarrow 0$ to be known in order to compute the solution. For each value of C_0 and k_{bp}/D_{eff}, there is only one unique value of $\frac{dC}{dr}$ at $r \rightarrow 0$ that satisfies the boundary condition $C(r \rightarrow \infty) = 0$. This value is obtained iteratively using a user written routine in MATLAB.

The model is fitted to data using nonlinear regression analysis with a simplex search method. The following model was used to characterize the residual error:

$$\log(Y_i) = \log(C_i) + \epsilon_i \quad (7)$$

where C_i is the i^{th} concentration predicted by the model and Y_i is the measured concentration. The residual departure of the model from the i^{th} observation is represented by ϵ_i. Values of ϵ_i are assumed to be independently and normally distributed, with mean zero and variance σ^2. Confidence intervals for the parameter estimates were obtained from so-called Log Likelihood profiles. The difference between the Log Likelihood at a certain value of the parameters and the Log Likelihood at the true parameter value is approximately χ^2 distributed. An approximate 95% marginal confidence interval for a specific parameter is the set of all parameter values for which −2 times the Log Likelihood is not more than 3.84 points higher than −2 times the Log Likelihood at the parameter estimate provided by the regression analysis.

In vivo *recovery*

In the present experiments $C_{t,\,dial}$ depends directly on the *in vivo* recovery of the detection probe (the factor between the measured concentrations in the dialysate and the true concentrations in brain ECF at the site of measurement) as well as indirectly by the *in vivo* loss from the infusion probe (by affecting C_0). The values for these recoveries may deviate from the recovery determined in an *in vitro* experiment (Bungay *et al.*, 1990). In principle, the normalized C_{ss} values as a function of r, determined by the two-dimensional model, provides a means to estimate *in vivo* recovery values. If the recovery were 100%, the value of C_0 should be 1, because C_0/C_0 should be 1 by definition. Any deviation of the value of C_0 from 1 reflects the *in vivo* recovery as a result of the individual contributions of the infusion and the detection probe. *In vivo* recovery values for the individual probes have been calculated by taking the square root of the deviation factor, assuming an equal contribution of each probe. These values were used to calculate cortical brain ECF concentrations ($C_{t,\,brain\,ECF}$) from the $C_{t,\,dial}$. The shape of the C versus r curve is independent of the actual recovery; the actual recovery scales the C versus r curve by the same amount at each value of r. Thus, the parameter estimates of k_{bp}/D_{eff} are not affected by the actual recovery and the fact that $C_{t,\,dial}$ is used in the fitting procedure instead of $C_{t,\,brain\,ECF}$.

Results

Profiles

Upon subsequent local infusions of acetaminophen at concentrations of 0.16, 0.51 and 1.3 mM, quite different concentrations were observed at the detection probe at interprobe distances of approximately x = 1 mm ($n=7$) and x = 2 mm ($n=7$). A representative individual $C_{t,\,brain\,ECF}$ of acetaminophen (Figure 1a) shows that three clearly different steady-state levels were obtained. For atenolol the same experiments were performed after infusion of 3.9, 5.9 and 7.8 mM. A representative $C_{t,\,brain\,ECF}$ of locally infused atenolol is shown in Figure 1b. As examplified by this figure, steady-state was not reached for atenolol concentrations within 230 min. The C_{ss} values have, therefore, been estimated by extrapolation, based on the equation $C_t = C_{ss}(1\text{-}e^{-kss.t})$ (Methods, equation 1). For both drugs the $C_{t,\,brain\,ECF}$ that resulted from the first infusion were used to calculate the kinetic parameters as presented in Table 1.

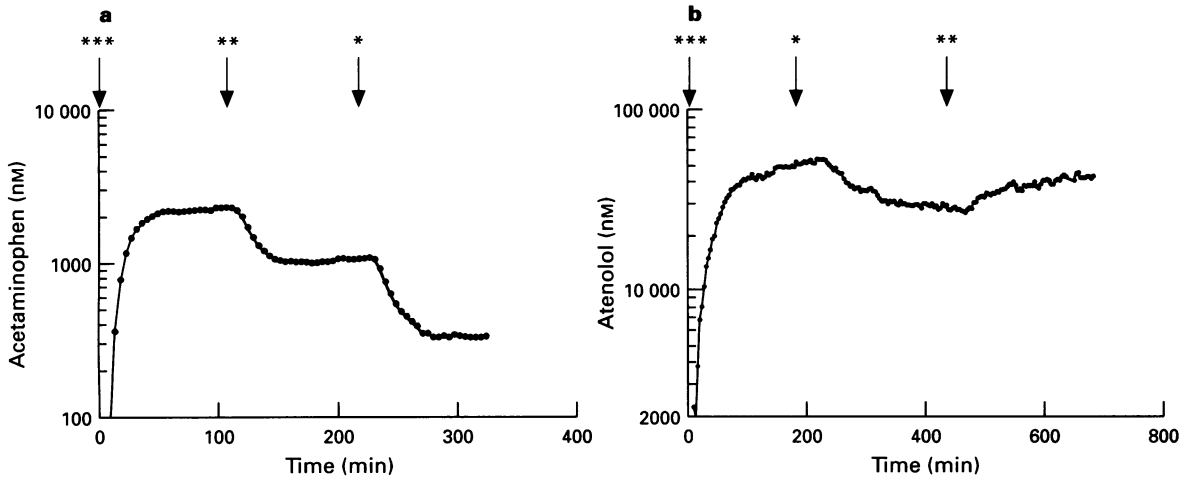

Figure 1 Individual $C_{t,\ brain\ ECF}$ as obtained with intracerebral microdialysis of rat brain cortex, during local infusion of subsequently the concentrations 1.3 (***), 0.5 (**), and 0.16 (*) mM for acetaminophen (a) or 7.8 (***), 3.9 (*), and 5.9 (**) mM for atenolol (b); the distance between infusion and detection probe was about 1 mm in these experiments.

Table 1 Disposition parameters of acetaminophen and atenolol in rat brain cortex after local infusion at two interprobe distance classes

	Interprobe distance r=1	*Interprobe distance* r=2
Acetaminophen	$n=7$	$n=7$
mean x (mm)	1.1±0.1	2.0±0.2
$T_{50\%}$ (min)	26±0.4	41±5.3
k_{ss} (min^{-1})	0.041±0.005	0.031±0.004
Atenolol	$n=6$	$n=7$
mean x (mm)	1.0±0.1	1.7±0.1
$T_{50\%}$ (min)	90±13	114±14
k_{ss} (min^{-1})	0.013±0.004	0.0068±0.0001

Values are mean±s.e.mean.
$T_{50\%}$ = time needed to reach 50% of steady state concentration.
k_{ss} = rate constant to reach steady state condition.

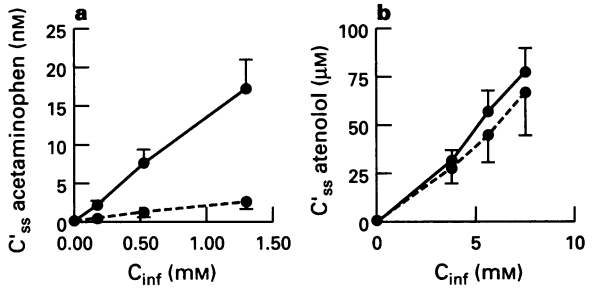

Figure 2 Relationship between mean $C_{ss,\ dial}$ values and the C_{inf} for (a) acetaminophen at r±1 mm and r±2 mm (r=0.998 and 0.996 respectively) and for (b) atenolol at r±1 mm and r±2 mm (correlation coefficient=0.994 and 0.997 respectively). Values are mean± s.e.mean.

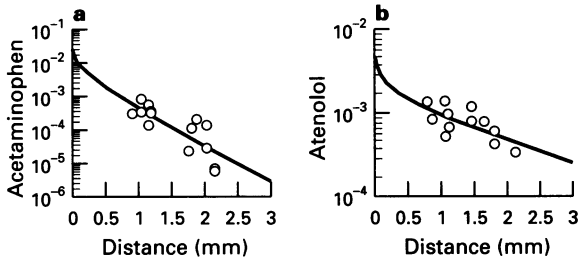

Figure 3 Spatial distribution of acetaminophen (a) and atenolol (b). The lines represent the calculated fits of the two-dimensional model. Each point represents the mean value of three (experimentally obtained and normalized) C_{ss} values, resulting from three different infusion concentrations (a: $n=14$; b: $n=13$).

Spatial distribution of atenolol and acetaminophen

A linear relation was found between the concentration in the infusion probe and the detection probe (Figure 2), so, a significant concentration-dependency did not occur. Therefore, the normalized $C_{ss,\ dial}$ values were used in calculations on spatial distribution. In the present experimental setup, the spatial distribution of both drugs is two-dimensional. In order to describe the normalized $C_{ss,\ dial}$ as a function of the interprobe distance r, a two-dimensional analysis has been performed on the data obtained for acetaminophen ($n=14$) and atenolol ($n=13$). Figure 3 depicts the experimental values as well as the calculated fits. It appears that the normalized $C_{ss,\ dial}$ for the more lipophilic drug acetaminophen declines more rapidly with increasing r than that for atenolol. The distance for which the normalized $C_{ss,\ dial}$ equals 1% of the C_0 is 1.1 mm for acetaminophen and about 4.5 mm for atenolol. Table 2 shows the estimation of k_{bp}/D_{eff} (cm^{-2}) from these plots. Despite the variability it can be seen that the mean value for acetaminophen, 550 (170–1240), is significantly higher than that for atenolol, 12 (0–73).

In vivo *recovery*

The C_0 value reflects the *in vivo* recovery. Assuming equal contributions of one individual probe, the *in vivo* recovery will be 20% (8–54%) for acetaminophen and 8% (7–13%) for

Table 2 Parameter estimates (95% confidence intervals) obtained from the two-dimensional model, applied to the spatial concentration distribution at steady-state conditions

	Acetaminophen	*Atenolol*
k_{bp}/D_{eff} (cm^{-2})	550 (170–1240)	12 (0–73)
C_0	0.039 (0.0066–0.294)	0.0068 (0.0042–0.0165)

k_{ss} = rate constant to reach steady state conditions.
D_{eff} = effective diffusion constant of drug through brain ECF.
C_0 = concentration of drug in brain ECF at r=0.

Table 3 Recovery values and [approximate 95% confidence intervals] of acetaminophen and atenolol as obtained for a microdialysis probe with a 8 mm semipermeable part

Recovery	*Acetaminophen*	*Atenolol*
In vitro	18% ($n=3$) [15–21%]	10% ($n=3$) [7–13%]
In vivo	20% ($n=14$) [8–54%]	8% ($n=13$) [7–13%]

atenolol. It appeared that the mean *in vivo* recovery values are similar to the ones obtained *in vitro* (Table 3).

Discussion and conclusions

This study was performed to gain further insight in the potential utility of *in vivo* intracerebral microdialysis as a technique to obtain information on the local disposition kinetics of drugs within the cortical brain. Infusion of acetaminophen or atenolol via the infusion probe resulted in steady-state concentrations, determined by the use of detection probes implanted in parallel which were dependent on the drug as well as on the interprobe distance. The results demonstrate that intracerebral microdialysis measures local concentrations which can be used to estimate parameters like k_{ss}, k_{bp}/D_{eff} and *in vivo* recovery.

Among the *in vivo* techniques applied to obtain information about drug transport into the brain, autoradiography and serial CSF sampling have been used. Quantitative autoradiography provides information on distribution of a radioactively labelled compound in the brain at one point in time, without discrimination between bound and unbound levels, or parent compounds and metabolites. By serial CSF sampling, drug distribution into the CSF can be measured as a function of time; however, regional differences in drug distribution in the brain cannot be assessed. Intracerebral microdialysis is an *in vivo* technique in which a semipermeable membrane is perfused with a physiological solution. This allows the continuous monitoring of the free drug in the extracellular space in a selected part of the brain, and also offers the opportunity to introduce drugs into brain tissue by infusion (Quan & Blatteis, 1989).

Previous microdialysis studies on transport of atenolol and acetaminophen into the brain have already shown that well-defined and reproducible concentration-time profiles can be obtained from brain ECF after systemic administration of these drugs (De Lange *et al.*, 1994). However, upon intracerebroventricular (i.c.v.) administration significant inter-animal differences in the cortical dialysate concentrations of acetaminophen were obtained, while for atenolol the concentrations were below the detection limit of the assay. Upon i.c.v. administration of these drugs, the profiles obtained in CSF after serial CSF sampling resulted in more or less reproducible concentration-time profiles (Van Bree *et al.*, 1989). In an earlier study, clear differences in the brain dialysate concentrations of atenolol were found between the two hemispheres after unilateral hyperosmolar BBB opening (De Lange *et al.*, 1995), suggesting that with microdialysis local concentration differences within the brain can be studied. If intracerebral microdialysis could indeed provide information on local differences in the concentration of a compound in the interstitial fluid, it would be a very powerful tool to investigate BBB transport at different sites within the brain.

Regional differences in concentrations of drugs have been demonstrated in extensive studies on the penetration of various substances into brain parenchyma following ventriculocisternal perfusion (Pollay & Kaplan, 1970; Patlak & Fenstermacher, 1975; Blasberg *et al.*, 1975) or microperfusion (Sendelbeck & Urquhart, 1985). The findings of these studies show that the more lipophilic compounds do not reach substantial concentrations beyond 1 or 2 mm of the ventricular outlines while substances with low rates of transcapillary exchange penetrated more deeply into the brain. Furthermore the depth of penetration increased with time during the period of study.

In the present study local infusion of atenolol (log P = −1.78) or acetaminophen (log P = 0.25) was used to create different regional concentration gradients in the brain. Individual $C_{t,\,dial}$ of acetaminophen or atenolol were evaluated after the subsequent local infusion of three different C_{inf} at an

Table 4 Several compounds and their experimentally determined values for: log P (octanol/water); distance for which $C_x/C_0 = 0.01$ ($x_{0.01,\,cm}$); capillary exchange coefficient (k_{bp}); effective diffusion coefficients (D_{eff}); and the ratio k_{bp}/D_{eff}

Compound	*log P*	$x_{0.01}$	k_{bp} (min^{-1})	D_{eff} ($cm^2\ s^{-1}$)	k_{bp}/D_{eff} (cm^{-2})
Sucrose	−3.67[g]	1.0[h]	$2.1*10^{-3}$ [d] $>2.8*10^{-3}$ [c]	$3.10*10^{-6}$ [a]	11 >9
Urea	−2.80[g]	0.4[a,i]			
Methotrexate	−2.50[g]	1.0[e] 0.2[c]		$0.59*10^{-6}$ [c]	
Ethylene glycol	−1.90[g]	0.4[j]	$2.3*10^{-2}$ [c] $6.9*10^{-2}$ [c]		
Atenolol	−1.78[k]	0.45			12
Creatinine	−1.77[g]	0.5[a] 0.3[a,i]		$2.20*10^{-6}$ [a] $0.89*10^{-6}$ [i]	
Hydroxyurea		0.31[c]		$1.50*10^{-6}$ [c]	
Acetaminophen	0.25[k]	0.11			550
Antipyrine	0.38[k]	0.4[e]			
BCNU	1.54[g]	0.12[c]	0.7[c]		

Values as obtained from: [a]Patlak & Fenstermacher (1975), [b]Fenstermacher *et al.* (1974), [c]Blasberg *et al.* (1975), [d]Dykstra *et al.* (1992), [e]Sendelbeck & Urquhart (1985), [f]Rapoport *et al.* (1979), [g]Levin (1980), [h]Fenstermacher *et al.* (1970), [i]Pollay & Kaplan (1970), [j]Fenstermacher *et al.* (1974), [k]van Bree *et al.* (1988).

interprobe distance between 1 and 2 mm. From these profiles k_{ss} values could be determined, which largely depend on the elimination rates of the drugs from the brain or overall elimination rate from the body. The k_{ss} values found in this study indeed appeared to be similar to the elimination rate values as found in a previous study (De Lange *et al.*, 1994). In principle, time-dependent changes in *in vivo* recovery could have affected the concentration-profiles (Morrison *et al.*, 1992) and therefore also the k_{ss} values. However, in these particular experiments, the *in vitro* and *in vivo* recovery values for both drugs were similar, indicating that the estimation of the k_{ss} values has not been influenced significantly by this factor.

$C_{ss, dial}$ values for acetaminophen were always achieved within the infusion period, while for atenolol these values had to be estimated by extrapolation. For acetaminophen and atenolol the relation between C_{inf} and $C_{ss, dial}$ was linear, and therefore $C_{ss, dial}$ values, after being normalized for C_{inf}, could be used in further calculations. At steady-state, the spatial concentration-gradient within the cortical brain is much steeper for acetaminophen than for atenolol (Figure 3). These gradients lie well within the range of those found for other compounds, reflected by the distance where 1% of the infusion concentration was found (Table 4). The concentration-gradient is the result of elimination processes from the brain like transcapillary transport from brain to blood, metabolism, and effective diffusion. For atenolol and acetaminophen no metabolism in brain has been reported, so the spatial profile will reflect transcapillary transport and effective diffusion (including all components that affect the diffusion process through brain ECF). For the description of the spatial distribution of atenolol and acetaminophen a two-dimensional analysis of the normalized $C_{ss, dial}$ versus r data was applied. From these plots the ratio of k_{bp} over D_{eff} could be calculated, which appeared to be 550 and 12 (cm^{-2}) for acetaminophen and atenolol respectively. The range of D_{eff} values is not as large as for k_{bp} values for small compounds (Table 4). This indicates that the ratio value being considerably larger for acetaminophen (46 times) is probably due to the higher transcapillary transport of acetaminophen, as expected on the basis of lipophilicity differences.

Spatial distribution in this study may have been influenced by the alteration of tissue characteristics due to implantation of the probe. Using the intracerebral microdialysis probe as an infusion device, Dykstra *et al.* (1992) have assessed the spatial distribution of sucrose with autoradiography. Theoretical calculations indicated an increase in extracellular volume. However, those experiments were started immediately after the insertion of the microdialysis infusion probe. Based on reports on histological, functional metabolic, and blood flow changes caused by the probe insertion, it appeared that the optimal time for starting microdialysis experiments is 8–48 h after implantation (Benveniste *et al.*, 1984; 1987; Westerink & de Vries, 1988). The experiments in the present study were performed 24 h after probe insertion, and its anticipated that at this time the influence of oedema around the microdialysis probe is minimal.

The estimation of *in vivo* recovery is a general problem of intracerebral microdialysis (Bungay *et al.*, 1990). The value of C_0 would be 1 if the recovery had been 100%. Any deviation of the value of C_0 from 1 reflects the *in vivo* recovery as a result of the individual contributions of the infusion and the detection probe. If both probes contribute equally to the *in vivo* recovery, the mean value for *in vivo* recovery of one of the two probes can be calculated. In this experimental design the *in vivo* and *in vitro* recovery values appeared to be more or less equal (Table 3).

The results of this study show that intracerebral microdialysis is able to detect local differences in drug concentrations, as well as differences in the spatial distribution of drugs with different lipophilicity in accordance with earlier reports using other *in vivo* techniques. The study demonstrates the potential use of intracerebral microdialysis to obtain pharmacokinetic parameters of drug distribution in brain by means of monitoring local concentrations of drugs in time.

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