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# Population Pharmacokinetic Modeling of Blood-Brain Barrier Transport of Synthetic Adenosine $A_1$ Receptor Agonists

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## ABSTRACT

A population pharmacokinetic model is proposed for estimation of the brain distribution clearance of synthetic $A_1$ receptor agonists in vivo. Rats with permanent venous and arterial cannulas in combination with a microdialysis probe in the striatum received intravenous infusions of 8-methylamino-$N^6$-cyclopentyladenosine (MCPA) and 2′-deoxyribose-$N^6$-cyclopentyladenosine (2′-dCPA) (10 mg $kg^{-1}$). The clearance for transport from blood to the brain was estimated by simultaneous analysis of the blood and extracellular fluid concentrations using a compartmental pharmacokinetic model. The proposed pharmacokinetic model consists of three compartments describing the time course of the concentration in blood in combination with three compartments for the brain extracellular fluid concentrations. The blood clearance was 7.4 ± 0.5 for MCPA and 7.2 ± 1.4 ml $min^{-1}$ for 2′-dCPA. The in vivo microdialysis recoveries determined by the dynamic-no-net-flux method were independent of time with values of 0.21 ± 0.02 and 0.22 ± 0.01 for MCPA and 2′-dCPA, respectively. The values of the intercompartmental clearance for the distribution from blood to brain were 1.9 ± 0.4 versus 1.6 ± 0.3 $\mu$l $min^{-1}$ for MCPA and 2′-dCPA, respectively. It is concluded that on basis of the novel six-compartment model precise estimates of the rate of brain distribution are obtained that are independent of eventual differences in systemic exposure. The low brain distribution rates of MCPA and 2′-dCPA were consistent with in vitro tests. Furthermore, a slow elimination from the brain compartment was observed, indicating that the duration of central nervous system effects may be much longer than expected on the basis of the terminal half-life in blood.

Blood-brain barrier (BBB) transport is a major determinant of the effect of CNS active drugs. This transport is determined by: 1) the morphology and functionality of the brain capillaries and 2) the physicochemical characteristics of the drug. Specifically, the transport of hydrophilic drugs is limited due to the presence of tight junctions between the capillary endothelial cells (Pardridge, 1991; Madara, 1998). Characterization of the BBB transport is therefore an important aspect of the development of CNS-active drugs.

At present, there are several approaches to the characterization of the BBB transport that can broadly be divided into three categories: 1) in vitro assays, 2) in situ perfusion techniques, and 3) in vivo methods. Blood-brain barrier transport is often studied in vitro in cocultures of brain-capillary-endothelial cells and astrocytes (Rubin et al., 1991; Gaillard et al., 2001). This approach is attractive because it allows identification of the specific mechanisms (i.e., transporters) that may be involved in the transport. However, the extrapolation from especially these novel in vitro models, consisting of a coculture of brain-capillary-endothelial cells and astrocytes, to the in vivo situation has not been established. This is important since both the passive permeability and the expression of specific transporters in in vitro models can be quite different from the in vivo situation. Another limitation is that factors such as the binding to plasma proteins and the cerebral perfusion rate, which may influence the brain uptake, are not considered. This underscores the need for detailed in vivo studies on BBB transport.

To study drug transport to the brain in vivo, frequently destructive sampling techniques have been applied. Nowadays, intracerebral microdialysis is an established technique for studying the physiology, pharmacology, and pathology of

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**ABBREVIATIONS:** BBB, blood-brain barrier; CNS, central nervous system; ECF, extracellular fluid; MCPA, 8-methylamino-$N^6$-cyclopentyladenosine; 2′-dCPA, 2′-deoxy-$N^6$-cyclopentyladenosine; CHA, $N^6$-cyclohexyladenosine; GR79236, $N^6$-[1*S*,*trans*-2-hydroxycyclopentyl]-adenosine; HPLC, high-pressure liquid chromatography.

a wide range of low-molecular weight substances in the brain extracellular fluid (ECF) (Bourne, 2003). Intracerebral microdialysis is also increasingly applied in pharmacokinetic studies to characterize drug transport to the brain in vivo (Malhotra et al., 1994; De Lange et al., 1999; Hammarlund-Udenaes, 2000). The latter approach offers the advantage of the ability to estimate the pharmacologically active unbound concentration close to the site of action in individual rats (De Lange et al., 1997; Elmquist and Sawchuk, 1997). Recently, intracerebral microdialysis has also been applied in pharmacokinetic/pharmacodynamic modeling. Specifically, a compartmental model has been proposed to describe the BBB transport of morphine-6-glucuronide to account for the delay of the antinociceptive effect relative to the corresponding plasma concentrations (Bouw et al., 2001). However, in most studies published to date, no formal pharmacokinetic analysis has been applied. Typically, the extent of BBB transport is characterized nonparametrically on basis of area under the curve values in plasma and ECF. As a consequence, no specific estimate of the rate of BBB transport (i.e., the intercompartmental clearance) is obtained. This complicates the comparison of the in vivo BBB transport characteristics of drugs and the examination of in vitro/in vivo correlations of BBB transport. This concerns specifically the BBB transport of drugs with widely different systemic pharmacokinetic properties and situations in which the plasma kinetics have changed as a result of, for example, the coadministration of inhibitors of specific transporters such as P-glycoprotein.

$A_1$ adenosine agonists are potentially useful drugs for the treatment of a variety of CNS disorders including sleep disturbances (Strecker et al., 2000), epilepsy (Malhotra and Gupta, 1997), cerebral ischemia and stroke (von Lubitz, 1999, 2001), and neuropathic pain (Sawynok, 1998). The chemical structure of $A_1$ receptor agonists is characterized by the presence of a ribose moiety. Consequently, these molecules are quite hydrophilic, which restricts their transport across the BBB. Recently, we have characterized the BBB transport characteristics of a series of $A_1$ adenosine agonists in an experimental in vitro model of the BBB consisting of a coculture bovine brain capillary endothelial cells and rat astrocytes as well as in an in situ brain perfusion model. These investigations revealed highly restricted transport of these compounds across the BBB. Furthermore, it was shown that these compounds are largely transported by passive diffusion and that observed differences in the diffusion can be explained by their physicochemical characteristics (Schaddelee et al., 2003). The objective of the present investigation was to determine, in a strict quantitative manner, the clearance for brain distribution of synthetic $A_1$ receptor agonists in vivo, by population pharmacokinetic analysis of the time course of the concentration in blood and brain ECF. The selective $A_1$ receptor partial agonists C8-methylamino-$N^6$-cyclopentyladenosine (MCPA) and 2′-deoxy-$N^6$-cyclopentyl adenosine (2′-dCPA) were chosen as model drugs on basis of previous investigations, demonstrating significant differences in BBB transport between both agonists (Schaddelee et al., 2003).

## Materials and Methods

### Chemicals

$N^6$-Cyclohexyladenosine (CHA) was purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). 2′-dCPA, MCPA, and GR79236 ($N^6$-[1S,*trans*-2-hydroxycyclopentyl]-adenosine) were kindly provided by GlaxoSmithKline (Uxbridge, Middlesex, UK). Ethyl acetate was purchased from Baker Chemicals (Deventer, The Netherlands) and distilled prior to use. Acetonitrile (DNA synthesis grade) was obtained from Biosolve (Valkenswaard, The Netherlands). Methanol [high-pressure liquid chromatography (HPLC) grade] was obtained from Rathburne (Walkersburn, UK). All other chemicals were of analytical grade (Baker Chemicals). Water was used from a Milli-Q system (Millipore SA, Molsheim, France).

### Animals

Male Wistar rats (Broekman B.V., Someren, The Netherlands) weighing between 250 and 300 g were housed in groups for 10 days, under standard environmental conditions (ambient temperature 21°C, 60% humidity, 12-h light/dark cycle, with lights on at 7:00 AM). The animals had free access to food (laboratory chow; Hope Farms, Woerden, The Netherlands) and acidified water. After surgery, the animals were housed individually in plastic cages for 1 week.

### Surgical Procedures

The rats were anesthetized with an intramuscular injection of 0.1 mg $kg^{-1}$ of Domitor (medetomidine hydrochloride; Pfizer, Capelle a/d IJssel, the Netherlands) and 1 mg $kg^{-1}$ Ketalar (Ketaminebase; Parke Davis, Hoofddorp, The Netherlands). Cannulas were implanted into the right jugular vein for drug administration and into the left femoral artery for blood sampling. The arterial cannula consisted of 4.5-cm polyethylene tubing (i.d. 0.28, o.d. 0.61 mm; Portex Limited, Hythe, UK) heat-sealed to 18-cm polyethylene tubing (i.d. 0.58, o.d. 0.96 mm; Portex Limited). The venous cannula consisted of 12-cm polyethylene tubing (i.d. 0.58, o.d. 0.96 mm). The cannulas were subcutaneously tunneled to the back of the neck. To prevent clotting, the cannulas were filled with 25% (w/v) polyvinylpyrrolidone (Brocacef, Maarssen, The Netherlands) solution in saline (0.9%) containing heparin (50 IU/ml; pharmacy at Leiden University Medical Centre, Leiden, The Netherlands). For probe implantation, the rats were placed in a stereotaxic frame, and the skull was exposed. A small hole was drilled to allow implantation of a microdialysis guide cannula (CMA/12; Aurora Borealis Control B.V., Schoonebeek, The Netherlands) in the anterior striatum relative to bregma (AP, 0.8; L, 2.7; V, −3.5). Two support screws were placed to hold the guide, which was glued to the skull with dental cement (dental acrylic cement, Howmedia simplex rapid + methylacrylate; Drijfhout, Amsterdam, The Netherlands).

### Experimental Procedures

**Microdialysis Experiment.** At the start of the experiment, the microdialysis probe (CMA/12, membrane length of 4.0 mm; Aurora Borealis Control B.V.) was inserted into the guide cannula. The inflow tubing was connected to a syringe pump (Beehive; Bas Technicol, Congleton, UK). The probe was perfused with artificial extracellular fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.2 mM ascorbic acid in 2 mM phosphate buffer, pH 7.4; Moghaddam and Bunney, 1989) at a flow rate of 2 $\mu$l $min^{-1}$. The outlet tubing was connected to a microsamples collector (Univentor 820; Antec, Leiden, The Netherlands). After 2 h of equilibration, the rats received an intravenous bolus infusion in 15 min of either 10 mg/kg MCPA or 2′-dCPA via the jugular vein cannula. A total number of between 13 and 20 dialysate fractions (10 to 30 min each) were collected, and 20 arterial blood samples (20 to 200 $\mu$l) were drawn for determination of the concentration of MCPA and 2′-dCPA, respectively. The blood samples were directly hemolyzed in glass tubes containing 400 $\mu$l of water and stored at −20°C until analysis.

**In Vivo Recovery.** To determine the drug concentration in the ECF surrounding the microdialysis probe, the in vivo recovery was determined using the dynamic-no-net-flux method (Olson and Justice, 1993). The experiments were conducted in a manner similar to the microdialysis experiments described above, except that the probe

was now perfused with MCPA concentrations of 10 or 30 ng ml$^{-1}$ and 2′-dCPA concentrations of 62.5, 125, or 250 ng ml$^{-1}$. Each group consisted of three to four rats.

**Plasma Protein Binding.** In the microdialysis experiments, an additional blood sample of 350 $\mu$l was taken at the end of the infusion for the determination of the plasma-to-blood concentration ratio and the free drug concentration in plasma. The total blood concentration was determined in a 20-$\mu$l blood sample, which was directly hemolyzed with 400 $\mu$l of water. The remaining blood was centrifuged at 4°C to separate the plasma. A sample of 20 $\mu$l was retained for analysis, and the remaining plasma was transferred into a Centrifree centrifugal filter device (Millipore Corporation, Billerica, MA) and centrifuged for 10 min at 1100*g* at 37°C to obtain 40 $\mu$l of plasma ultrafiltrate. The samples were stored at −20°C until analysis.

## Drug Analysis

**Blood Samples.** *MCPA.* The blood, plasma, and ultrafiltrate samples were analyzed by a previously described reversed-phase HPLC method (Van Schaick et al., 1997). Briefly, CHA (50 $\mu$l, 6 $\mu$M) was added to the blood samples as internal standard. The samples were extracted with 5 ml of ethyl acetate. After centrifugation, the organic layer was transferred into clean tubes, and 500 $\mu$l of water and 50 $\mu$l of sodium hydroxide (3 M) were added. The samples were extracted for the second time, and the organic layer was separated from the aqueous layer. The organic layer was evaporated to dryness under reduced pressure at 37°C. The residue was dissolved in 100 $\mu$l of mobile phase, and 75 $\mu$l was injected onto the chromatographic system. The chromatographic system consisted of an LC-10AD HPLC pump (Shimadzu, Kyoto, Japan), a WISP-712 autosampler (Waters, Milford, MA), and a spectroflow 757 variable wavelength UV detector (Applied Biosystems, Foster City, CA) set at 269 nm. The output signal of the UV detector was processed with a C-R3A reporting integrator (Shimadzu) in the peak height mode. For the analysis, a stainless steel Microsphere C18 3-$\mu$m cartridge-column (10- × 4.6-mm i.d.) was used. The mobile phase consisted of a mixture of acetate buffer (50 mm, pH 4.0) and acetonitrile in the ratio 79:21 (v/v). TEA was added to the mobile phase (100 $\mu$l l$^{-1}$). At a flow rate of 0.5 ml min$^{-1}$, the retention times were 9.1 and 15.2 min for MCPA and CHA, respectively. The calibration curves were analyzed under weighted linear regression (weight factor: $1/y^2$). The detection limit (signal-to-noise ratio of 3) was 15 ng ml$^{-1}$ for a 50-$\mu$l blood sample. The extraction recovery was 84%. The within- and between-day variations were determined in a concentration range of 50 to 2000 ng ml$^{-1}$ and were less than 5.2 and 6.7%, respectively.

*2′-dCPA.* Blood, plasma, and ultrafiltrate samples were analyzed by a previously described reversed-phase HPLC method (Mathôt et al., 1995). Briefly, CHA (50 $\mu$l, 6 $\mu$M) was added to the blood samples as internal standard. The samples were alkalinized with 50 $\mu$l of sodium hydroxide (3 M) and extracted with 5 ml of ethyl acetate. The organic layer was transferred to clean tubes and evaporated to dryness under reduced pressure at 37°C. The residue was dissolved in 100 $\mu$l mobile phase, and 75 $\mu$l was injected into the HPLC. The same chromatic system was used as in the MCPA analysis. The mobile phase consisted of a mixture of acetate buffer (25 mM, pH 4.0), methanol, and acetonitrile (56:40:4 v/v). At a flow rate of 0.5 ml min$^{-1}$, the retention times were 7.9 and 13.1 min for 2′-dCPA and CHA, respectively. The calibration curve was analyzed under weighted linear regression (weight factor: $1/y^2$). The detection limit (signal-to-noise ratio of 3) was 2.5 ng ml$^{-1}$ for a 100-$\mu$l blood sample. The extraction recovery was 70%. The within- and between-day variations were determined in a concentration range of 100 to 2500 ng ml$^{-1}$ and were less than 1.6 and 9.6%, respectively.

**Dialysate Samples.** The dialysate samples were analyzed by HPLC with tandem mass spectrometry. Calibration standards were prepared in water. The dialysate samples and calibration standards were transferred into a 96-well plate and dried under nitrogen at 40°C. The residues were dissolved in 100 $\mu$l of a mixture of water and methanol (95:5 v/v) containing 100 ng ml$^{-1}$ GR79236 as internal standard. A volume of 50 $\mu$l was injected into the LC system. HPLC was performed on a Hewlett Packard 1100 instrument (Hewlett Packard, Waldbronn, Germany). Chromatography was performed on a C18 column (50- × 2.1-mm i.d.; 5 $\mu$M particle size) (Capital HPLC, Broxburn, UK) at a flow rate of 0.4 ml min$^{-1}$. The mobile phase consisted of 2 solvents: water + 0.1% formic acid (A), and 100% acetonitrile + 0.1% formic acid (B). The profile was 0 to 2 min 100% A; 2 to 3 min linear gradient to 90% B; 3 to 3.5 min 90% B; 3.5 to 3.7 min linear gradient to 100% A; and 3.7 to 5 min 100% A. Mass spectrometry was performed on a PE-Sciex API2000 instrument (PerkinElmerSciex Instruments, Boston, MA) equipped with a turbo ion spray source used in the positive mode. Detection by tandem mass spectrometry was based on precursor ion transitions to the strongest intensity. Instrumental conditions were optimized to yield best sensitivity. The detection limits for a 10-$\mu$l ECF sample were for both compounds 0.5 ng ml$^{-1}$. The within- and between-day variations were determined in a concentration range of 2 to 50 ng ml$^{-1}$ and were less than 3.9 and 9% and 6.9 and 13% for MCPA and 2′-dCPA, respectively.

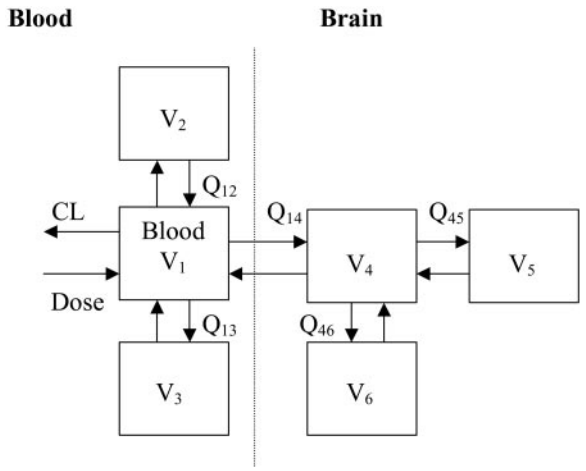


**Fig. 1.** The population pharmacokinetic model for synthetic adenosine $A_1$ receptor agonists comprising three compartments describing the pharmacokinetics in blood and three compartments describing the pharmacokinetics in brain ECF.

## Data Analysis

**Population Pharmacokinetic Model.** To estimate the intercompartmental clearance for the transport from blood to the brain, the compartmental model depicted in Fig. 1 was fitted to the blood and ECF concentration versus time profiles. In this approach, the blood and ECF data from all individual rats were simultaneously analyzed while explicitly taking into account both the interindividual variability in the model parameters as well as interindividual residual error (Schoemaker and Cohen, 1996). All fitting procedures were performed in the NONMEM (nonlinear-mixed-effect-modeling) software (GloboMax, Hanover, MD) using the subroutine ADVAN 7, which is a general linear model that uses the numerical solution of the differential equations. Three compartments for description of the kinetics in blood in combination with three additional compartments for the kinetics in the brain were selected on the basis of the Akaike information criterion (Akaike, 1974). The blood and ECF concentration versus time data were modeled according to the following differential equations.

$$\frac{dA_1}{dt} = R_{iv} - K_{12}\cdot A_1 + K_{21}\cdot A_2 - K_{13}\cdot A_1 + K_{31}\cdot A_3 - K_{14}\cdot A_1 + K_{41}\cdot A_4 - \mathrm{CL}\cdot\frac{A_1}{V_1} \quad (1a)$$

$$\frac{dA_2}{dt} = K_{12} \cdot A_1 - K_{21} \cdot A_2 \quad (1b)$$

$$\frac{dA_3}{dt} = K_{13} \cdot A_1 - K_{31} \cdot A_3 \quad (1c)$$

$$\frac{dA_4}{dt} = K_{14} \cdot A_1 - K_{41} \cdot A_4 - K_{45} \cdot A_4 + K_{54} \cdot A_5 - K_{46} \cdot A_4 + K_{64} \cdot A_6 \quad (1d)$$

$$\frac{dA_5}{dt} = K_{45} \cdot A_4 - K_{54} \cdot A_5 \quad (1e)$$

$$\frac{dA_6}{dt} = K_{46} \cdot A_4 - K_{64} \cdot A_6 \quad (1f)$$

in which $R_{iv}$ is the zero-order infusion rate, $A_l$ is the amount in compartment $l$, $K_{mn}$ is the first-order transfer rate constant from compartment $m$ to compartment $n$, CL is the clearance from the central compartment, and $V_1$ is the volume of distribution of the central compartment. The rate constants for distribution between the compartments $m$ and $n$ were determined from the intercompartmental clearance ($Q$) and compartment volume ($V$) according to:

$$K_{mn} = \frac{Q_{m+1}}{V_m} \quad (2a)$$

$$K_{nm} = \frac{Q_{m+1}}{V_n} \quad (2b)$$

In the modeling of both compounds, the volumes of the compartment 4 ($V_4$) and 6 ($V_6$) were assumed to be equal. Furthermore, for 2′-dCPA, the volumes of the compartment 1 ($V_1$) and 3 ($V_3$) were assumed equal, whereas for MCPA this was also assumed to be the case for the compartments 1 ($V_1$) and 2 ($V_2$). The parsimonious model yielded the same minimum value of the objective function as the full model.

Interindividual variability on the parameters was modeled according to an exponential equation; it was assumed that the parameters were log-normally distributed:

$$\theta_i = \theta \cdot \exp(\eta_i) \quad (3)$$

where $\theta$ is the population mean parameter value, $\theta_i$ is the individual parameter (e.g., $V_1$, CL, $Q_{14}$), and $\exp(\eta_i)$ is a random term from a normal distribution with mean zero and variance $\omega^2$. The $\eta_i$ values quantify the deviation of the individual parameters from the population mean; therefore, the variance $\omega^2$ associated with parameter $\theta$ provides a measure of interindividual variation in $\theta$, which relates to the biological variation and experimental errors. The interindividual variation was estimated for the following model parameters: CL, $V_1$, $V_2$, and $Q_{14}$. The residual error was characterized by a combination of a proportional and additive error model:

$$Cm_{ij} = C_{ij} \cdot (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad (4)$$

where $C_{ij}$ is the $j$th blood or ECF concentration for the $i$th individual predicted by the model, $Cm_{ij}$ is the measured blood or ECF concentration, and $\epsilon_{1ij}$ and $\epsilon_{2ij}$ account for the residual deviance of the model predicted value from the observed concentration. The values for $\epsilon$ are normally distributed with mean zero and variance $\sigma^2$.

The first-order Bayesian estimation method implemented in the NONMEM software was used to calculate population and individual parameter estimates. All fitting procedures were performed on an IBM-compatible personal computer (Pentium, 133 MHz) running under Windows NT using the Microsoft FORTRAN Powerstation 4.0 compiler with NONMEM version IV, level 2 (double precision) and Visual NONMEM version 2.2.2 (RDPP, Montpellier, France).

**In Vivo Recovery.** The in vivo recovery was estimated on the basis of the linear relationship between the perfusate concentration ($C_{in}$) and the perfusion concentration minus the dialysate concentration ($C_{in} - C_{out}$). For the estimation of the in vivo recovery, a population approach was applied utilizing all information of multiple observations for each individual rat. The data were analyzed in NONMEM (GloboMax) using the following linear model:

$$y = ax + b \quad (5)$$

where $x$ is the perfusate concentration ($C_{in}$), $y$ is the perfusion concentration minus the dialysate concentration ($C_{in} - C_{out}$), $a$ is the in vivo recovery, and $b$ is the $y$-ordinate intercept. Time was included in the analyses as covariate. The data were analyzed both by subject and time to estimate the intrasubject and intratime variability.

**Statistical Analysis.** The pharmacokinetic parameter estimates were compared statistically using the one-way $t$ test. A significance level of 5% was selected. All data are reported as mean ± S.E., unless indicated otherwise.

## Results

The novel six-compartment model was able to describe the pharmacokinetics of MCPA and 2′-dCPA in blood and brain ECF. Three compartments were required for description of the time course of the concentrations in blood while an additional three compartments were required to describe the kinetics in the brain. The blood concentration-time profiles following intravenous infusion of MCPA and 2′-dCPA are shown in Fig. 2. The post hoc and population estimates for the blood pharmacokinetics, the blood-to-plasma concentration ratio, and the free fraction in plasma are listed in Table 1. The clearance and volume of distribution were similar between MCPA and 2′-dCPA; however, the compounds differed in the plasma-to-blood concentration ratio and the free fraction in plasma. The plasma-to-blood concentration ratio and the free fraction in plasma were 1.3 ± 0.1, 0.59 ± 0.06 versus 0.28 ± 0.05, 0.62 ± 0.05 for MCPA and 2′-dCPA, respectively.

The in vivo recovery was determined by the dynamic-no-net-flux method for both MCPA and 2′-dCPA. A population approach was used for the estimation of the in vivo recovery on the basis of a linear model describing the relationship between $C_{in} - C_{out}$ as a function of $C_{in}$. The results of the in vivo recovery experiment for MCPA and 2′-dCPA are shown in Fig. 3. The straight lines in Fig. 3 are the population predictions, and the slopes are the population estimates for the in vivo recovery. The population estimates of the in vivo recovery were 0.211 ± 0.019 and 0.219 ± 0.014 of MCPA and 2′-dCPA, respectively. The individual recovery estimates versus time patterns for MCPA and 2′-dCPA are depicted in Fig. 4. In the analysis, time was included as covariant and appeared not to be statistically significant. The intrasubject variability was not statistically significant from zero. Therefore, the brain ECF concentrations were calculated as the ratio of the dialysate concentrations and the population predicted in vivo recovery.

The brain ECF concentration-time profiles after intravenous infusion of MCPA or 2′-dCPA are shown in Fig. 5. The post hoc and population prediction estimates for the brain ECF pharmacokinetic parameters are summarized in Table 2. No statistically significant differences in brain pharmacokinetic parameters were found between MCPA and 2′-dCPA. Previous investigations have demonstrated that the binding

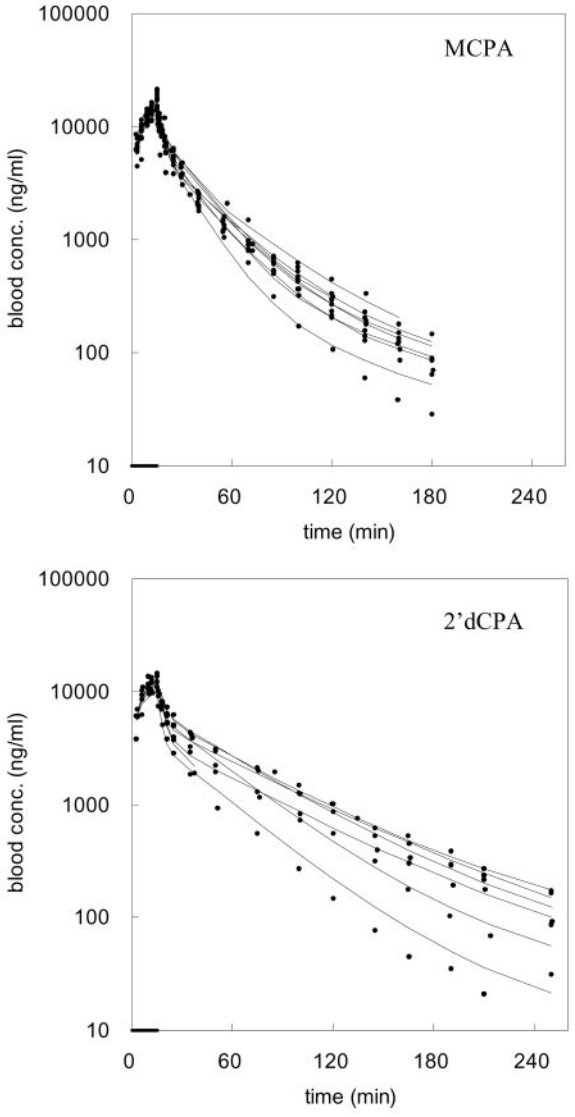


**Fig. 2.** The individual plasma concentration-time profiles after intravenous administration of 10 mg/kg MCPA or 2′-dCPA in 15 min to rats. ●, individually observed concentrations; solid lines, individual predictions based on the population six-compartment pharmacokinetic model.

to blood constituents restricts the brain uptake (M. P. Schaddelee, K. D. Read, C. G. J. Cleypool, A. P. IJzerman, M. Danhof, A. G. De Boer, unpublished observations). The unbound intercompartmental clearance from blood to brain ($Q_{14,u}$) was calculated as the ratio of the total intercompartmental clearance ($Q_{14}$) divided by the plasma-to-blood ratio and the free fraction in plasma. The values were 6.24 ± 2.78 and 4.29 ± 1.29 $\mu$l min$^{-1}$ for MCPA and 2′-dCPA, respectively. The volumes of distribution of the brain compartments were high compared with those of the peripheral compartments. The values of the hypothetical volume of distribution of the brain compartment were 280 ± 67 and 181 ± 39 ml for MCPA and 2′-dCPA, respectively. These high values of the volume of the brain compartment reflect significant binding of the compounds to brain tissue components. In Fig. 6, the concentrations in blood and ECF for a typical rat were simulated up to 12 h after the start of the infusion. Figure 6 illustrates the large differences in slope of the terminal concentration-time profiles in ECF compared with blood. The elimination out of the brain for both compounds appears to be much slower than the elimination from blood.

## Discussion

$A_1$ receptor agonists are potential useful drugs for the treatment of a variety of CNS disorders (Malhotra and Gupta, 1997; Sawynok, 1998; von Lubitz, 1999, 2001; Strecker et al., 2000). Due to presence of a ribose moiety, these agonists are quite hydrophilic, which restricts the transport across the BBB. Recently, we have characterized the BBB transport of a series of $A_1$ receptor agonists in an experimental in vitro model of the BBB consisting of a coculture of brain capillary endothelial cells and astrocytes and in in situ perfusion studies. These investigations revealed that the BBB transport of these compounds is restricted. It was also demonstrated that these compounds are largely transported by passive diffusion and that observed differences in diffusion can be explained in part by their physicochemical characteristics (Schaddelee et al., 2003).

The purpose of this study was to characterize, in strict quantitative manner, the BBB transport of synthetic $A_1$ receptor agonists in vivo, by intracerebral microdialysis in combination with population compartmental pharmacokinetic modeling. Two prototype $A_1$ receptor agonists were selected on the basis of previous investigations in which it was demonstrated that significant differences in BBB transport exist between both agonists (Schaddelee et al., 2003).

MCPA and 2′-dCPA showed similar blood concentration-time profiles. No statistically significant differences were found in the pharmacokinetic parameters describing the plasma concentration versus time profiles of both drugs, with the exception of the distribution into red blood cells and the plasma protein binding. MCPA binds to blood constituents with a plasma-to-blood concentration ratio of 1.3 ± 0.14. For 2′-dCPA, the plasma-to-blood concentration ratio was 0.59 ± 0.06. The free fractions in plasma were 0.28 ± 0.05 and 0.62 ± 0.05 for MCPA and 2′-dCPA, respectively. The values of the pharmacokinetic parameters in the present investigation are similar to those in previous studies (Mathôt et al., 1995; Van Schaick et al., 1997).

An important issue that needs to be addressed using the microdialysis technique is the in vivo recovery, which describes the relationship between the measured dialysate concentrations and the "true" ECF concentrations. The in vivo recovery is not only dependent on the probe characteristics but also on periprobe processes like intraextracellular exchange of the compound and tissue damage (Bungay et al., 1990). Furthermore, the in vivo recovery may change with time (Morrison et al., 1992).

In the present study, a novel approach was applied to determine the in vivo recovery: population nonlinear mixed effects modeling of results obtained with the dynamic-no-net-flux method (Olson and Justice, 1993). A unique feature of this approach is that it allows not only the influence of time dependence to be determined but also estimation of the intrasubject variability in in vivo recovery. A linear model successfully described the relationship between $C_{in} - C_{out}$ and $C_{in}$ with the slope of this relationship reflecting the in vivo recovery. No statistically significant difference in in vivo recovery was found between MCPA and 2′-dCPA, with population mean estimates of 0.21 ± 0.02 and 0.22 ± 0.01,

TABLE 1

Blood pharmacokinetic parameter estimates after intravenous administration of 10 mg $kg^{-1}$ MCPA or 2′-dCPA

Presented are both the population mean estimates ± S.E. with intersubject variability between brackets and the mean estimates ± S.E. of the individual post hoc Bayesian estimates.

| | MCPA | 2′-dCPA |
|---|---|---|
| CL (ml $min^{-1}$) | | |
| Population mean | 7.44 ± 0.47 (18%) | 7.2 ± 1.4 (48%) |
| Mean of post hoc Bayesian estimates | 7.70 ± 1.42 | 6.04 ± 2.27 |
| $Q_{12}$ (ml $min^{-1}$) | | |
| Population mean | 0.715 ± 0.29 | 11.0 ± 1.6 (31%) |
| Mean of post hoc Bayesian estimates | 0.715 | 11.9 ± 3.1 |
| $Q_{13}$ (ml $min^{-1}$) | | |
| Population mean | 7.79 ± 2.2 | 0.405 ± 0.15 |
| $V_1$ (ml) | | |
| Population mean | 57.8 ± 9.8 (22%) | 60.6 ± 4.0 |
| Mean of post hoc Bayesian estimates | 60.1 ± 9.67 | |
| $V_2$ (ml) | | |
| Population mean | 81.3 ± 18 (30%) | 203 ± 23 (25%) |
| Mean of post hoc Bayesian estimates | 87.1 ± 22 | 183 ± 36 |
| $V_3$ (ml) | | |
| Population mean | 81.3 ± 18 (30%) | 60.6 ± 4.0 |
| Mean of post hoc Bayesian estimates | 87.1 ± 22 | |
| Plasma-to-blood concentration ratio | 1.25 ± 0.14* | 0.59 ± 0.06* |
| Free fraction in plasma | 0.28 ± 0.05* | 0.62 ± 0.05* |
| $N$ | 9 | 7 |

* $p < 0.05$.

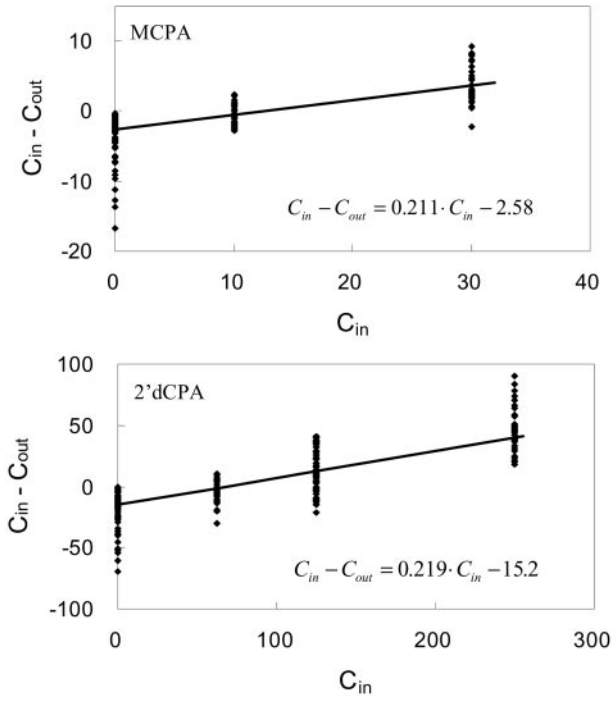


**Fig. 3.** The perfusion concentration ($C_{in}$) versus the perfusion concentration minus the dialysate concentration ($C_{in} - C_{out}$) for MCPA or 2′-dCPA. The solid line represents the population prediction based on the linear model describing the relationship $C_{in} - C_{out}$ as a function of $C_{in}$. The slope of this relationship represents the in vivo recovery.

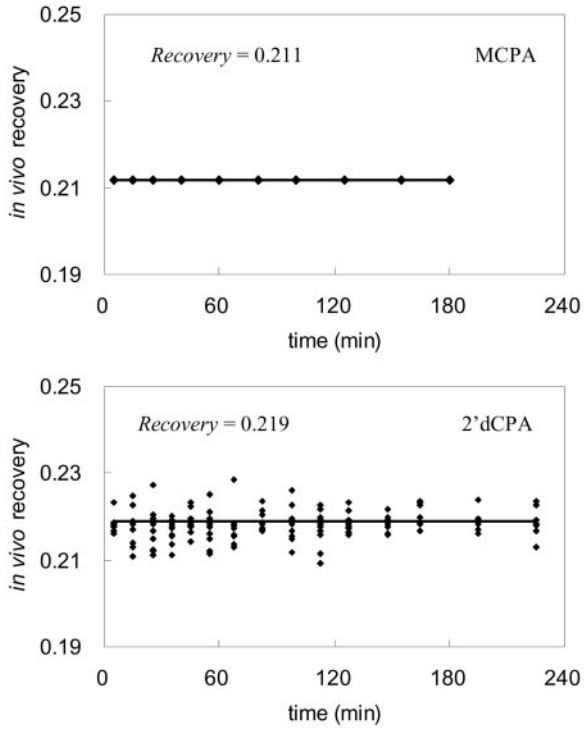


**Fig. 4.** The individual in vivo recovery estimates versus time for MCPA or 2′-dCPA. The straight line represents the population prediction. The slope of the straight line was not significantly different from zero, indicating that the in vivo recovery is independent of time.

respectively. For MCPA and 2′-dCPA both the intratime and the intrasubject variability were not significantly different, statistically, from zero. Since for both MCPA and 2′-dCPA there was neither a significant time dependence nor a significant intrasubject variability in in vivo recovery, the mean population estimates of the in vivo recovery were used for the estimation of the periprobe in vivo ECF concentrations from the microdialysate concentrations.

The proposed six-compartment model accurately described the concentration versus time profiles of both compounds in blood plasma as well as brain extracellular fluid. This model was established on the basis of an iterative analysis of the data using a variety of different models. In this analysis, it was specifically determined whether simplified models [i.e., one- or two-compartment model(s)] could describe the data equally well; however, analysis of the data with the simplified models was not justified as reflected in a considerable loss of goodness-of-fit. In the analysis, saturable brain equilibration kinetics was also considered, by incorporation of a Michaelis-Menten expression in the intercompartmental

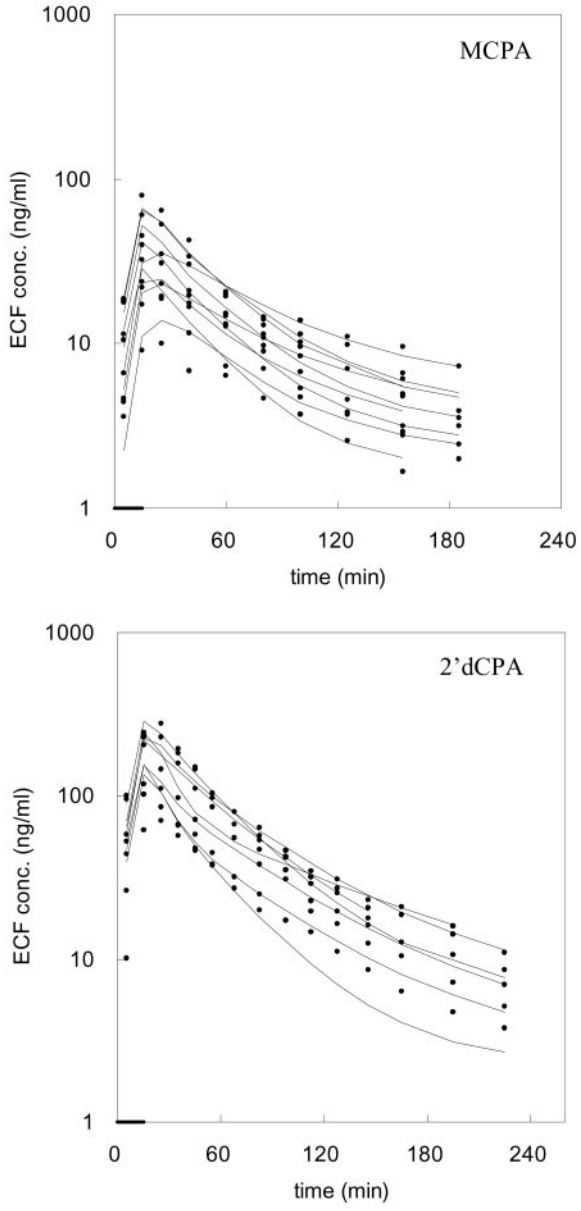


**Fig. 5.** The individual ECF concentration-time profile after intravenous administration of 10 mg/kg MCPA or 2′-dCPA to rats. ●, individually observed concentrations; solid lines, individual predictions based on the population six-compartment pharmacokinetic model.

clearance between blood plasma and brain. This did not result in an improvement of the goodness-of-fit, indicating that saturable processes do not contribute significantly to the overall transport. This is consistent with previous observations in the in vitro BBB model (Schaddelee et al., 2003) and in in situ perfusion studies (M. P. Schaddelee, K. D. Read, C. G. J. Cleypool, A. P. IJzerman, M. Danhof, A. G. De Boer, unpublished observations).

Another objective of this investigation was to explore the in vitro/in situ/in vivo correlation of the BBB transport of $A_1$ receptor agonists. The extrapolation from novel in vitro BBB models to in vivo models has not been established. This is important since both the passive permeability and the expression of specific transporters in in vitro models can be quite different from the in vivo situation. In addition, factors such as protein binding and the cerebral perfusion rate, which might influence the brain uptake, are not considered. The comparison of in vitro and in vivo data requires a formal quantitative pharmacokinetic analysis of the in vivo data, allowing precise estimation of the in vivo distribution clearance between blood and brain.

The brain ECF concentration profiles of MCPA and 2′-dCPA had similar profiles, albeit that higher concentrations were observed for 2′-dCPA than for MCPA. The intercompartmental clearance from the central blood compartment to the central brain compartment ($Q_{14}$) were 1.94 ± 0.37 and 1.64 ± 0.48 $\mu$l min$^{-1}$ for MCPA and 2′-dCPA, respectively. Recent investigations using in situ brain perfusion have provided experimental evidence that $A_1$ receptor agonists are low-extraction ratio compounds ($E < 0.01$) with respect to the brain uptake (M. P. Schaddelee, K. D. Read, C. G. J. Cleypool, A. P. IJzerman, M. Danhof, A. G. De Boer, unpublished observations). The observations in the present investigation confirm this since the estimated values of the brain distribution clearance are indeed much lower than the reported value of the brain perfusion in vivo of 2.2 ml min$^{-1}$ g$^{-1}$ in rats (De Visscher et al., 2003). The BBB transport for low-extraction ratio compounds is related to the unbound blood concentration instead of the whole-blood concentration (Levy and Moreland, 1984). Therefore, the unbound intercompartmental clearance from blood to brain ($Q_{14,u}$) was calculated for both drugs. No statistically significant difference was found between MCPA and 2′-dCPA with values of 6.24 ± 2.78 and 4.29 ± 1.29 $\mu$l/min for MCPA and 2′-dCPA, respectively. The intersubject variability in the intercompartmental clearance was 39 and 27% for MCPA and 2′-dCPA, respectively. For both agonists, large differences in the terminal concentration-time profiles in ECF compared with blood were observed. The elimination out of the ECF was much slower for both compounds than the elimination out of blood, which can be explained by the high volume of distribution of the brain compartments, reflecting binding to brain tissue constituents This observation is of considerable interest since this might explain why duration of action in the CNS could last much longer than expected on the basis of the terminal half-life in blood.

Previous in vitro and in situ studies have demonstrated that, in general, $A_1$ receptor agonists are poorly transported across the BBB (Schaddelee et al., 2003). Interestingly, similar differences in clearances and ranking were found in the in situ perfusion studies as in vitro transport studies (M. P. Schaddelee, unpublished observations). The intercompartmental clearances of MCPA and 2′-dCPA in the present study are similar to previously found values using in situ brain perfusion (MCPA, 4.5 ± 2.1; 2′-dCPA, 22.0 ± 2.8 $\mu$l min$^{-1}$ g$^{-1}$; M. P. Schaddelee, K. D. Read, C. G. J. Cleypool, A. P. IJzerman, M. Danhof, A. G. De Boer, unpublished observations) albeit that the clearance for 2′-dCPA is somewhat higher in the in situ brain perfusion study, compared with the presently obtained value on the basis of microdialysis. An important issue in this respect is that in situ brain perfusion is a single-pass technique. As a consequence, the in vivo brain distribution clearance obtained in this manner represents only the uptake of the drug in the brain. In contrast, the clearance obtained by microdialysis considers the data during the infusion and elimination phase and, thereby, reflects the bidirectional distribution to and from the brain. As such, the results obtained with the microdialysis technique

TABLE 2

ECF pharmacokinetic parameter estimates after intravenous administration of 10 mg $kg^{-1}$ MCPA or 2′-dCPA

Presented are both the population mean estimates (with intersubject variability between brackets) and the mean of the individual post hoc Bayesian estimates. $p < 0.05$.

| | MCPA | 2′-dCPA |
|---|---|---|
| $Q_{14}$ ($\mu$l $min^{-1}$) | | |
| Population mean | 1.94 ± 0.37 (39%) | 1.64 ± 0.48 (27%) |
| Mean of post hoc Bayesian estimates | 2.06 ± 0.73 | 1.62 ± 0.44 |
| $Q_{14,u}$ ($\mu$l $min^{-1}$) | | |
| Population mean | 6.24 ± 2.78 | 4.29 ± 1.29 |
| $Q_{45}$ ($\mu$l $min^{-1}$) | | |
| Population mean | 0.342 ± 0.05 | 0.0631 ± 0.018 |
| Mean of post hoc Bayesian estimates | 0.342 | |
| $Q_{46}$ ($\mu$l $min^{-1}$) | | |
| Population mean | 0.167 ± 0.04 | 0.279 ± 0.10 |
| $V_4$ (ml) | | |
| Population mean | 4.91 ± 1.61 (72%) | 0.278 ± 0.080 |
| Mean of post hoc Bayesian estimates | 6.55 ± 4.7 | |
| $V_5$ (ml) | | |
| Population mean | 280 ± 67 | 181 ± 39 |
| $V_6$ (ml) | | |
| Population mean | 4.91 ± 1.61 (72%) | 0.278 ± 0.080 |
| Mean of post hoc Bayesian estimates | 6.55 ± 4.7 | |
| $n$ | 9 | 7 |

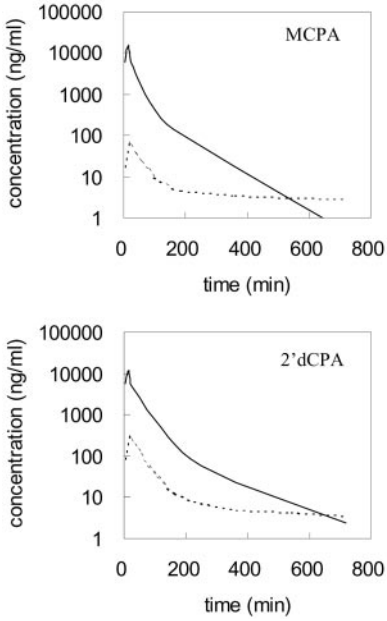


**Fig. 6.** Simulated average blood and ECF concentration-time profiles after intravenous administration of 10 mg/kg MCPA or 2′-dCPA for a typical rat. Solid line, blood; dotted line, ECF concentration-time profile. The data were simulated up to 12 h after the start of infusion.

are more representative of the processes that determine the onset and the duration of the pharmacological response in vivo. An important feature of the microdialysis technique in combination with compartmental modeling for qualification of the brain equilibration kinetics is that the approach is universally applicable, specifically also to compounds, which are chemically unrelated.

In conclusion, on the basis of the novel six-compartment model, estimates of the rate of in vivo BBB transport of synthetic $A_1$ receptor agonists were obtained. The intercompartmental clearances of MCPA and 2′-dCPA were similarly low and consistent with the results of previous in vitro tests. The compartmental pharmacokinetic analysis used in this study has the advantage over traditional nonparametric methods that this approach quantifies the rate of BBB transport independent of differences in systemic exposure. This allows comparison of in vivo with in vitro data but also comparison between compounds that have different systemic pharmacokinetic properties or in situations where plasma kinetics has changed. Furthermore, the results obtained on the basis of in vivo microdialysis in combination with population pharmacokinetic modeling consider the bidirectional distribution to and from the brain and are, therefore, representative of the process that determines the time course of the drug effect.

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