NEUROPHARMACOKINETICS OF A NEW α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLE PROPIONIC ACID (AMPA) MODULATOR, S18986 [S]-2,3-DIHYDRO-[3,4]CYCLOPENTANO-1,2,4-BENZOTHIAZIDINE-1,1-DIOXIDE, IN THE RAT

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ABSTRACT:

The aim of our study was to determine the neuropharmacokinetics of S18986, a new positive allosteric modulator of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type receptors, in the rat. We focused on its blood-brain barrier (BBB) uptake and on its brain intra- and extracellular fluid (bICF-bECF) partitioning. BBB transport of S18986 was measured using the in situ brain perfusion technique. bECF concentrations were determined by microdialysis in the two effector areas, i.e., frontal cortex (FC) and dorsal hippocampus (DH), and blood samples were collected simultaneously through a femoral catheter. Cerebrospinal fluid and brain tissue concentrations were determined using a conventional pharmacokinetic approach. Using all the experimental data, pharmacokinetic modeling was applied to describe the S18986 blood-brain disposition. The brain uptake clearance of S18986 was found to be high, about 20 μl s⁻¹ g⁻¹. Terminal half-lives were similar in plasma and brain, at around 1 h. Experimental and predicted blood and brain concentrations were a good fit with the pharmacokinetic model, which assumed first-order rate constants at each interface. Ratios of bECF to the unbound plasma area under the curve (AUC) were 0.24 in FC and 0.25 in DH, whereas ratios of bICF/plasma AUC were 1 in FC and 1.5 in DH. We conclude that despite the ratio of bECF/plasma AUC below 1, there is nevertheless an elevated BBB uptake of S18986. This can be explained by the S18986 nonhomogenous bECF/bICF partitioning, since S18986 mainly distributes into hippocampal bICF. This illustrates the importance of taking bECF/bICF partitioning into account when interpreting the neuropharmacokinetics of a drug.

Materials and Methods

Animals. Male Wistar rats (Iffa Credo, L’Arbresles, France) weighing 200 to 300g were used. They had free access to standard laboratory food and water and were maintained under a 12-h light/dark cycle at 22 ± 1°C. The animals were housed under these conditions for at least 5 days before being used. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals.

ABBREVIATIONS: S18986, (S)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiaizidine-1,1-dioxide; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; BBB, blood-brain barrier; bECF, brain extracellular fluid; CSF, cerebrospinal fluid; bICF, brain intracellular fluid; HPLC, high-performance liquid chromatography; FC, frontal cortex; DH, dorsal hippocampus; AUC, area under the curve.
Laboratory Animals (National Institutes of Health publication #85–23, revised 1985).

Drugs and Chemicals. S18986 (Institut de Recherches Internationales Servier, Courbevoie, France) was dissolved in purified water for oral administration. [14C]S18986 was provided by PerkinElmer Life and Analytical Sciences (Boston, MA). Its specific activity was 49 mCi mmol⁻¹, and its chemical purity was greater than 99% by thin-layer chromatography and nonchiral high-performance liquid chromatography (HPLC) and greater than 95% by chiral HPLC. [1H]Sucrose (377.4 GBq mmol⁻¹) was obtained from PerkinElmer Life and Analytical Sciences (Paris, France).

Chemicals were of HPLC grade. Ethanol, heptane, and dichloromethane were purchased from Fisher Scientific UK Ltd (Loughborough, UK); methanol and butan-1-ol were obtained from Romil Ltd (Cambridge, UK), and ammonium formate was obtained from VWR International Ltd (Merck House, Poole, UK). Liquid scintillation cocktails were purchased from Packard (Rungis, France).

In Situ Brain Perfusion Technique. Surgical Procedure and Perfusion Technique. The brain transport of [14C]S18986 was measured using the in situ brain perfusion method in rats as described previously by Takasato et al. (1994) and Smith (1996). Animals were anesthetized by intraperitoneal injection of diazepam (Roche, Neuilly-sur-Seine, France) and ketamine (Panhope, Paris, France) filled with heparin. Before insertion of the catheter, the common carotid artery was ligated on the proximal side. The external carotid and occipital arteries were also ligated. During surgery, body temperature was maintained from 37–38°C using a rectal thermistor connected to a temperature monitor. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000; Harvard Apparatus, Holliston, MA) and connected to the catheter. Before perfusion, the thorax of the animal was opened, the heart was cut, and carotid perfusion started immediately with a flow rate of 10 ml/min. The perfusion fluid consisted of bicarbonate-buffered physiological saline: 128 mM NaCl, 24 mM NaHCO₃, 4.2 mM KCl, 2.4 mM NaH₂PO₄, 1.5 mM CaCl₂, 0.9 mM MgCl₂, and 9 mM d-glucose. The solution was gassed with 95% O₂ and 5% CO₂ for pH control (7.4) and warmed to 37°C.

Briefly, the right common carotid was catheterized with polyethylene tubing (0.76 mm i.d. × 1.22 mm o.d.; Biotrol Diagnostic, Chennevières-les-Louvre, France) filled with heparin. Before insertion of the catheter, the common carotid artery was ligated on the proximal side. The external carotid and occipital arteries were also ligated. During surgery, body temperature was maintained from 37–38°C using a rectal thermistor connected to a temperature monitor. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000; Harvard Apparatus, Holliston, MA) and connected to the catheter. Before perfusion, the thorax of the animal was opened, the heart was cut, and carotid perfusion started immediately with a flow rate of 10 ml/min. The perfusion fluid consisted of bicarbonate-buffered physiological saline: 128 mM NaCl, 24 mM NaHCO₃, 4.2 mM KCl, 2.4 mM NaH₂PO₄, 1.5 mM CaCl₂, 0.9 mM MgCl₂, and 9 mM d-glucose. The solution was gassed with 95% O₂ and 5% CO₂ for pH control (7.4) and warmed to 37°C in a water bath. Compounds were added to perfusate at an adequate concentration. Three concentrations (1.23, 6.14, and 12.27 μM) of [14C]S18986 were tested. In each animal, [14C]S18986 was copurified with [1H]Sucrose (11.1 KBq ml⁻¹) as a physical integrity marker of the BBB. Perfusion was terminated by decapitation at 60 s. The brain was removed from the skull and dissected on ice. The right cerebral hemisphere was separated in three parts (hippocampus, frontal cortex, and the rest of the brain). Each of them was placed in tared vials and weighed. Aliquots of the perfusion fluid were also collected and weighed to determine tracer concentrations in the perfusate. Samples were digested in 2 ml of Solvable (Packard) at 50°C and mixed with 9 ml of Ultima gold XR scintillation cocktail (Packard). Dual label counting was performed simultaneously in a Packard Tri-Carb model 1900 TR (Packard).

Calculation of BBB Transport Parameters. Briefly, calculations were performed as previously described by Smith (1996). Brain vascular volume (Vuccess, μl g⁻¹) was estimated from the tissue distribution of [1H]Sucrose (which is known to diffuse very slowly across the BBB), using the following equation:

\[ V_{\text{success}} = X^*/C^* \]  

where \( X^* \) (dpm g⁻¹) is the amount of sucrose measured in the right brain hemisphere, and \( C^* \) (dpm μl⁻¹) is the concentration of labeled sucrose in the perfusion fluid. Transport across the BBB was expressed in terms of two parameters: the apparent volume of distribution (Vbrain) and the initial brain uptake clearance (Clup).

The apparent volume of distribution was calculated from the amount of radioactivity in the right brain hemisphere using the following equation:

\[ V_{\text{brain}} = X_{\text{brain}}/C_{\text{perf}} \]  

where \( X_{\text{brain}} \) (dpm g⁻¹) is the calculated amount of [14C]S18986 in the right cerebral hemisphere, and \( C_{\text{perf}} \) (dpm μl⁻¹) is the labeled tracer concentration in the perfusion fluid. Brain tissue radioactivity was corrected for vascular contamination with the following equation:

\[ X_{\text{brain}} = X_{\text{tot}} - V_{\text{success}} \cdot C_{\text{perf}} \]  

where \( T \) is the perfusion time (seconds). The perfusion time used was long enough to ensure that at least 40% total radioactivity in the tissue resided outside the vascular space [Xbrain ≥ 0.4 Xtot (Takasato et al., 1984)].

Brain Tissue and CSF Pharmacokinetics. A water suspension (1 ml) of S18986 was given by the oral route at the dose of 1 mg kg⁻¹. At 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after S18986 administration, 50 to 100 μl of CSF were taken by cisterna magna puncture, and rats were decapitated. Animals were anesthetized by intraperitoneal administration of diazepam (7.5 mg kg⁻¹) and ketamine (70 mg kg⁻¹) 15 min before CSF was taken. The brain was removed from the skull and dissected into three parts (frontal cortex, hippocampus, and the rest of the brain).

Three rats per time were investigated. CSF and brain samples were stored at −20°C until analysis.

Brain Extracellular Fluid (bECF) and Blood Pharmacokinetics. Microdialysis Procedure. Rats were anesthetized with diazepam (7.5 mg kg⁻¹ i.p.) and ketamine (70 mg kg⁻¹ i.p.), and the right femoral vein was cannulated with a silastic catheter for blood collection. The catheter was filled with heparin to prevent coagulation before the experiment. A subcutaneous tunnel allowed the catheter to be advanced to the level of the neck. Animals were then placed in a stereotaxic frame (David Kopf Instruments, Ronceau, France), and a CMA/12 guide cannula (CMA, Phymep, France) was implanted into the frontal cortex (FC) or the dorsal hippocampus (DH). Seven rats were used for each area. A hole was drilled in the skull at the appropriate coordinates for the frontal cortex (3.2 mm anterior to the bregma, 2.5 mm lateral to the sagittal suture, and −2 mm ventral to the skull surface) and the dorsal hippocampus (3.8 mm posterior to the bregma, 2.5 mm lateral to the sagittal suture, and 2.2 mm ventral to the skull surface), according to the atlas of Paxinos and Watson (1998). The guide cannula was held in place with dental cement and anchor screws. Rats were left to recover from femoral catheter and guide implantations for 24 h before the experiments.

The day of the experiment, a polycarbonate microdialysis probe (CMA/12, 3-mm length for frontal cortex, 2-mm length for dorsal hippocampus; CMA) was slowly inserted into the guide cannula 1 h before beginning the experiment and was connected to a perfusion pump (CMA/102; CMA). Brain physiological fluid (CMA fluid: 147 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, and 0.85 mM MgCl₂) was perfused through the microdialysis probe at the rate of 2.3 μl min⁻¹ for 2 h to attain equilibrium and permit collection of the blank samples. Then, S18986 was given by the oral route at the dose of 1 mg kg⁻¹. Dialysates were sampled every 30 min up to 3 h, and then every hour from 3 to 5 h after S18986 administration. Blood samples (180 μl) were collected at 0, 5, 8, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after S18986 administration. The
blood was centrifuged at 3000g for 5 min to collect plasma. Probe placement was controlled for each rat by microscopic examination of 120-µm brain slices at the end of the experiment.

In Vivo Microdialysis Probe Recovery. The retrodialysis method was performed using the radiolabeled molecule [14C]S18986. In a first set of experiments, in vivo probe recovery was investigated in a group of three rats for each area, i.e., FC and DH, that did not receive cold S18986 but instead a 6-h infusion of [14C]S18986. Dialysates were collected every 20 min. This enabled the stability of the recovery to be checked. In a second set of experiments, corresponding to the pharmacokinetic study, for each rat, at the end of the experiment, the in vivo recovery was measured by perfusing a tracer dose of 0.3 µM [14C]S18986 at the rate of 2.3 µl min⁻¹ through the microdialysis probe. Dialysates were collected every 20 min for 2 h. The recovery (R) was estimated by the loss of [14C]S18986 from the perfusate during the retrodialysis period (eq. 5):

\[ R = \frac{C_{in} - C_{out}}{C_{in}} \]  

where \( C_{in} \) is the [14C]S18986 concentration in the incoming perfusate, and \( C_{out} \) is the [14C]S18986 concentration in the outgoing dialysate. The bECF concentration of [14C]S18986 was obtained by correcting the dialysate concentration by the in vivo recovery, for each rat \( C_{bECF} = C_{dialysate}/R \), where \( C_{bECF} \) is the bECF concentration of [14C]S18986, \( C_{dialysate} \) is the measured concentration of [14C]S18986 in each dialysate sample, and \( R \) is the microdialysis probe recovery measured for each rat.

Brain Intracellular Fluid (bICF) Concentrations. bICF concentrations were calculated assuming that the total brain volume was the sum of the intra- and extracellular volumes (Scism et al., 2000) and that the extracellular volume was equal to 18% of the total brain volume (Bradbury, 1979; Rees et al., 1982). The bICF concentrations are then equal to:

\[ C_{bICF} = \frac{(C_{in} \times V_{tot}) - (C_{bECF} \times V_{bECF})}{V_{bICF}} \]  

where \( C_{bICF} \) is the brain intracellular concentration, \( C_{in} \) is the total brain concentration, \( C_{bECF} \) is the brain extracellular concentration, \( V_{tot} \) is the total brain volume, \( V_{bECF} \) is the brain extracellular volume, and \( V_{bICF} \) is the brain intracellular volume.

This estimation of bICF concentrations does not differentiate that part of drug freely distributed into brain intracellular fluid from that bound to brain cells. The total brain concentrations were first corrected by the cerebral volume, estimated at 30 µl g⁻¹ (Everett et al., 1956; Takasato et al., 1984).

Sample Analysis. Plasma and total brain tissue concentrations were assayed by HPLC with fluorometric detection. Samples were first subjected to liquid-liquid extraction. Briefly, 100 µl of sample was mixed with 300 µl of ethanol containing the internal standard (S18789; Institut de Recherches Servier) and centrifuged at 14,000 rpm for 3 min. Supernatants of plasma (350 µl) or brain tissue homogenate (300 µl) were taken, mixed with 350 µl of dichloromethane, and centrifuged at 14,000 rpm for 3 min. Five hundred microliters of the lower organic layer were carefully removed and placed into a conical glass grade glass HPLC vial and evaporated to complete dryness. Samples were reconstituted with 150 µl of butan-1-ol, and 6 µl (plasma) or 15 µl (brain tissue homogenate) were injected into the HPLC column [Hypersil 3 NH2–2 (APS-2) 150 × 4.6, 3 µm; Phenomenex, Torrance, CA]. The HPLC system consisted of a Waters 2790 pump and a tandem mass spectrometry detector (Quattro LC). Samples (40 µl) were mixed with the internal standard (S18789) and centrifuged at 3000 rpm for 3 min. Twenty microliters of supernatant was injected into an aqua C18 75 × 2 mm, 3 µm, column (Phenomenex). The gradient elution varied with time [0–5 and 8.1–10 min: A/B (90:10); 5–8 min: A/B (0:100)]. The mobile phase contained a mixture of A (10 mM ammonium formate) and B (methanol). The flow rate was 0.2 ml min⁻¹ and the column was maintained at 45°C. The retention times for S18986 were 6, 6.5, and 6.7 min in plasma, CSF and bECF, and brain tissue samples, respectively. The limits of quantification were 0.25, 2.5, and 10 ng ml⁻¹ in bECF-CSF, brain tissue and plasma, respectively. The inter- and intra-assay precisions, expressed as standard deviation over mean concentration, were 9.5 and 4.6%, respectively.

Pharmacokinetic Analysis. Data in each compartment (i.e., in bECF, bICF, CSF, and plasma) were analyzed using the average concentration at each time point.

Free plasma concentrations of S18986 were calculated based on in vitro and in vivo experiments, showing that the free plasma fraction was independent of the S18986 concentrations, constant over time and equal to 32.9 ± 4.8% in vivo (Institut de Recherches Internationales Servier, unpublished data).

Pharmacokinetic parameters in each biological fluid were first calculated using a model-independent method. The maximum concentration \( C_{max} \) and the time to reach \( C_{max} \) \( (T_{max}) \) were taken from experimental curves. The terminal half-lives were determined from the elimination rate constant \( K_e \), calculated by linear regression analysis of the last final points. The area under the concentration-time curve (AUC) was calculated by the trapezoidal method from 0 to 5 h and extrapolated from 5 h to infinity by dividing the concentration at 5 h by \( K_e \). The apparent clearance \( (CI) \) was calculated by dividing the dose by the AUC\(_{0\rightarrow\infty} \), and the apparent volume of distribution \( (V_d) \) was calculated by dividing the apparent clearance by \( K_e \). For corresponds to the bioavailability factor.

Pharmacokinetic Modeling. We first fitted the plasma data alone, to define the compartmental model and the plasma pharmacokinetic parameters, especially \( V_{1} \ F^{-1} \), \( K_{in} \) and \( K_{out} \), where \( V_{1} \ F^{-1} \) represents the apparent volume of the central compartment, \( K_{in} \) the absorption rate constant, and \( K_{out} \) the elimination rate constant. Based on visual and statistical criteria (see the statistical analysis section), we chose a one-compartmental model with first-order absorption and elimination rate constants.

Then we fitted all the experimental data together, according to the representative model shown in Fig. 2. The main hypothesis using this model was that \( K_e \) the absorption rate constant, and the intercompartmental rate constants \( K_{ij} \) to \( K_{ij} \) \((i,j = 1 to 5) \) were first-order, i.e., that no capacity-limited transfer occurs between any of the compartments.

The differential equations are as follows:

\[
\frac{dC_i}{dt} = \left[ (K_{12}C_1(t) \cdot V_2 + K_{22}C_2(t) \cdot V_2 + K_{13}C_1(t) \cdot V_3) / V_2 \right] - (K_{10} + K_{11} + K_{13})C_i(t) \\
\frac{dC_i}{dt} = -(K_{20} + K_{22})C_2(t) \\
\frac{dC_i}{dt} = \left[ (K_{12}C_1(t) \cdot V_1 + K_{32}C_3(t) \cdot V_3 + K_{23}C_2(t) \cdot V_4) / V_3 \right] - (K_{30} + K_{33} + K_{34})C_i(t) \\
\frac{dC_i}{dt} = \left( K_{32}C_3(t) \cdot V_1 + K_{42}C_4(t) \cdot V_4 + K_{34}C_3(t) \cdot V_4 \right) / V_4 \\
\frac{dC_i}{dt} = (K_{14}C_1(t) \cdot V_4 / V_4) - K_{14}C_i(t)
\]

where \( C_1 \), \( C_2 \), \( C_3 \), \( C_4 \), \( C_5 \), \( V_1 \), \( V_2 \), \( V_3 \), \( V_4 \) are, respectively, the concentrations and the apparent volumes in each compartment (Fig. 2). The initial parameters used for \( V_i \), \( K_{ij} \) and \( K_{10} \) were the values found with the individual plasma model (Table 1).

The intercompartmental constant \( K_{14} \) corresponds to the rate constant between brain and bECF, i.e., to the apparent initial brain uptake clearance \( (C_{Lup}) \) \( F^{-1} \) divided by the volume \( V_i \) \( F^{-1} \). We used as initial parameter for \( K_{14} \), the brain \( C_{Lup} \) of S18986 (divided by \( V_i \)), that we measured by the in situ brain perfusion technique, i.e., \( 20 \) µl g⁻¹ sec⁻¹.

The initial parameters used for the brain volumes \( (V_i, V_2, V_3) \) were literature values (Mahar Doan and Boje, 2000), i.e., 0.25, 0.29, and 0.99 ml, respectively.

Statistical Analysis. Brain Perfusion Experiment. Data are presented as mean ± standard deviation (S.D.) for four to six animals. Two-way (concentration and brain area) analysis of variance was used to identify significant
differences between groups, using SYSTAT version 8.0 (1998; SPSS Inc., Chicago, IL). The statistical significance was set at $p < 0.05$.

Pharmacokinetic Modeling. Models were fitted to the data using WinNonlin professional version 4.1 (Pharsight Corp., Mountain View, CA). The estimation method was the iterative reweighted last-squares weighting by the inverse of the predicted concentration squares, $1/C^2$.

The criteria used for the model evaluation were 1) the fit between observed and predicted concentrations, 2) the Akaike criterion, 3) the parameter’s coefficient of variation (CV, %), and 4) the weighted residual concentrations versus time plot. The Akaike criterion reflects the deviation between observed and estimated concentrations, since it is related to the objective function. This criterion must be as low as possible, indicating that the gap between estimated and observed concentration is the smallest. The parameter’s coefficient of variations must be below 20 to 30%, and the weighted residual concentrations versus time plot obtained between observed and predicted concentrations must be randomized above and below zero.

Results

In Situ Brain Perfusion. The initial brain uptake clearances ($Cl_{up}$) were not significantly affected either by the perfused S18986 concentration (1.23, 6.14, and 12.27 $\mu$M) ($p = 0.07$), which corresponded to current pharmacological systemic exposure to S18986, or by the brain area, i.e., the hippocampus, frontal cortex, and rest of the brain ($p = 0.6$), and were around 20 $\mu l$ s$^{-1}$ g$^{-1}$ (Fig. 3). In the same experiments, the integrity of the BBB was verified by measuring the brain vascular volume from the brain tissue distribution of $[3H]$sucrose, which does not measurably penetrate the BBB during brief periods of perfusion.

![FIG. 2. Multiple-compartment model, called the “complete model,” used for simultaneous brain and plasma pharmacokinetic analysis. All the transfer constants are assumed to be first-order.](image)

![TABLE 1 Unbound plasma pharmacokinetic parameters determined with the one-compartment model and with the ‘complete model’ shown in Fig. 2](table)

![FIG. 3. Initial brain uptake clearance, $Cl_{up}$ of $[^3H]$S18986 was measured by in situ brain perfusion in rats. Animals were perfused with $[^3H]$S18986 via the common carotid for 60 s. Three concentrations were tested. The solid, dotted, and open histograms represent the $Cl_{up}$ values for 1.23, 6.136, and 12.27 $\mu$M, respectively. Data are presented as the mean of four rats S.D. The results of the two-way analysis of variance show that the $Cl_{up}$ values were not affected either by the brain area ($p = 0.6$) or by the concentrations ($p = 0.07$).](image)
an average terminal half-life, which was found at around 1 h (Table 2). Figure 4 depicts the observed plasma concentrations versus time. In the brain microdialysis experiments, the dialysate concentrations were corrected by the in vivo probe recovery, 24.6 ± 3% in the frontal cortex and 19.2 ± 3.6% in the hippocampus. The terminal half-lives found in CSF, bECF, and bICF in frontal cortex and dorsal hippocampus were very close to the plasma terminal half-life (Table 2). By comparing the AUC ratio between bICF and bECF, we found that S18986 was distributed 4- and 6.2-fold more abundantly within the bICF than in the bECF spaces in frontal cortex and hippocampus, respectively. Finally, S18986 concentrations in bECF declined in parallel with those observed in bECF, CSF, and plasma (Fig. 5).

The area under the curve ratios of S18986 in bECF, CSF, and bICF to unbound plasma were 0.24, 0.4, and 1 in the frontal cortex and 0.25, 0.4, and 1.5 in the hippocampus, respectively (Table 2).

**Pharmacokinetic Modeling.** Based on the hypothesis (illustrated by the model depicted in Fig. 2) that only first-order pharmacokinetic processes determine the intercompartmental exchanges of S18986 between blood and brain at the BBB and within brain compartments, we found that the predicted concentrations in plasma, CSF, bECF, and bICF fitted the observed data (Figs. 4 and 5). The plasma pharmacokinetic parameters and their associated CV (%) obtained with the complete model are given in Table 1. The plasma parameters found are very close to those obtained by fitting plasma data alone, and the associated CV are below 30% (Table 1). In agreement with the experimental values, all the kinetics had parallel declines with terminal half-lives in each compartment of approximately one hour.

**Discussion**

Our findings allow us first to quantify the blood-brain uptake and the neuropharmacokinetics of a new positive allosteric modulator of AMPA receptors, S18986, and second to clarify more generally the question of blood-brain partitioning.

First, using the in situ brain perfusion technique, we show that in each brain area studied, i.e., frontal cortex and hippocampus, the brain uptake clearance (Cl_{up}) was constant within the range of concentrations tested (1.23 to 12.27 μM) at around 20 μl s⁻¹ g⁻¹. This value is nearer to the Cl_{up} observed for diazepam [45 μl s⁻¹ g⁻¹ (Takasato et al., 1984; Rousselle et al., 1998)] than for compounds being effluxed from brain to blood by the active transporter P-glycoprotein. Cl_{up} of the above compounds, such as morphine or vinblastine, were determined as 0.21 and 0.26 μl s⁻¹ g⁻¹, respectively, in our laboratory (Cisternino et al., 2001). Whatever the concentration tested, the brain extraction coefficient of S18986, taking diazepam as reference, is then 45%, meaning that the amount of S18986 extracted from the brain is high and proportional to the cerebral blood flow. Our results are in agreement with the results of an in vitro study showing that S18986 was highly permeable across Caco-2 cells (P = 25.10⁻⁶ cm s⁻¹ in both directions) and that S18986 was not a substrate of P-glycoprotein (C. O’Connell, R. Weaver, and T. Shepard, unpublished data). In view of this, the results of our study suggest that in the range of concentrations tested, the main component for Cl_{up} of S18986 is probably passive diffusion. Nevertheless, we cannot exclude that carrier-mediated processes, influxing or effluxing S18986 at the BBB, may interfere in parallel; however, if that is the case, such processes are probably not saturated within the concentration range of our study (1.23 to 12.27 μM) or make only a minor contribution, indicating an overall apparent first-order process for the brain uptake of S18986.

In view of the initial brain uptake clearance of S18986 at the BBB, we used this Cl_{up} value of 20 μl s⁻¹ g⁻¹ to fit the experimental plasma and brain data to the model shown in Fig. 2, which assumes that all the intercompartmental rate constants were first-order. As shown in Fig. 5, A and B, the model gives a good fit between the observed and predicted concentrations in each compartment, confirming our hypothesis. Indeed, if there had been any influx or efflux transport, i.e., if a zero-order process has been involved following transporter saturation, we could not have obtained such a good fit between our observed and predicted data, since we generated them using first-order equations.

Understanding the transport processes at the BBB is important because they frequently represent the limiting step for a drug to reach brain fluids and then the brain effector sites (Pardridge, 1999). That is why many experimental studies are investigating how to distinguish passive from active transport mechanisms at the BBB. Consequently, one often reads that examination of the ratio of bECF, usually measured by microdialysis, to unbound blood concentrations at steady-state gives information about the type of transport processes involved at the BBB. Hammarlund-Uleda et al. (1997) simulated plasma and bECF curves for several hypothetical CNS drugs using a two-compartment model (blood and brain), considering that the brain volume of distribution corresponds to the physiological bECF volume when no binding and no intracellular distribution occurs. They tested the effect on plasma and brain profiles of passive or active transports at the BBB and concluded that when bECF concentrations are parallel but lower than plasma concentrations, even if bECF bulk flow can in part explain this difference, in most cases, it is due to the involvement of an efflux process at the BBB. This group has thus reported for opioids like morphine and their metabolites, that since their bECF/unbound plasma steady-state concentration ratios were below one, active efflux occurred at the BBB (Bouw et al., 2000; Xie et al., 2000; Tunblad et al., 2003). However, the authors have not taken into account the bICF and/or CSF distribution of the opioids. Their conclusions were based on the fact that when the transport mechanism between two fluids separated by a membrane is independent of an active transport, the concentrations between the two fluids should tend to reach equilibrium. This has previously been extensively developed for describing the drug equilibrium between CSF and its unbound plasma levels. However, as in our study, the ratio of the drug concentration between plasma and CSF was not systematically equal to unity even if diffusion transport mainly determined the transport across the blood-cerebrospinal fluid barriers (Nau et al., 1993; Shen et al., 2004). In the overall question of the equilibrium between blood and brain, it is important to remember that bECF or CSF cannot be kinetically considered as representing the whole brain. In other words, the above authors, by concluding that a bECF/unbound plasma ratio below one means that an efflux transporter is active at the BBB, fail.
and microglial cell membranes (Declèves et al., 2000; Lee et al., 2001; Dallas et al., 2003), as well as on ependymal cells (Mercier et al., 2004). We investigated the partitioning between the bECF and bICF by combining the microdialysis and classical pharmacokinetic methods allowing the measurement of S18986 in the hippocampus and the frontal cortex, i.e., the two brain effector sites of S18986. The partitioning between bECF and CSF was studied using CSF data. We calculated the ratios between the bECF and unbound plasma areas under the curves and found them to be 0.24 in the frontal cortex and 0.25 in the hippocampus (Table 2). Based on the hypothesis that a drug, especially a lipophilic drug such as S18986, can partition between brain intra- and extracellular compartments and between bECF and CSF, we calculated the ratios that take into account the partitioning between bICF and CSF. The ratio of CSF to unbound plasma AUCs was 0.4, indicating that the distribution into CSF is approximately the same as that into bECF. However, the ratio of bECF to plasma AUC was equal to one in the frontal cortex and to 1.5 in the hippocampus (Table 2). The bICF/bECF AUC ratios (4 in frontal cortex and 6.2 in hippocampus) indicate that S18986 penetrates mainly into the brain cells or binds to their plasma membranes, and that S18986 seems to accumulate more in the hippocampus than in the cortex, suggesting that the hippocampus could be considered a more specific effector site than the cortex.

This allows us to propose that a bECF/unbound plasma AUC ratio below one does not systematically indicate that the molecule is effluxed from the brain at the BBB, but can be due to its partitioning from the bECF to the bICF spaces. The importance of taking into account the partition between bECF and bICF had been pointed out in a few works. One of the first was the study conducted by Wang and Welty (1996). The authors constructed a pharmacokinetic model similar to ours using first-order processes and applied it to gabapentin kinetics in brain and plasma. They showed that the ratio of bECF to plasma AUCs was below one (about 0.1) and explained this difference in part by the intracellular accumulation of gabapentin. Scism et al. (2000) showed in a rabbit study that a probenecid-sensitive transporter was involved in the valproic acid distribution at the level of the brain parenchymal cells. This was because the ratio at steady state of bICF to blood concentrations was 1.5-fold enhanced by the coadministration of probenecid, whereas the ratio of bICF to blood was unchanged by adding probenecid.

Understanding this partition can help to define the effector compartment. Indeed, the bECF or the bICF distribution of a drug can suggest that the receptors are mainly located in this compartment. In the case of S18986, we know that the receptors are chiefly located on the hippocampal cells. The fact that S18986 distribution in hippocampus bICF is greater than in bECF and other areas could suggest that hippocampal cells are the effector site.

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<th>bECF, hippocampus</th>
<th>bECF, frontal cortex</th>
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**TABLE 2**

Main pharmacokinetic parameters, calculated by a noncompartmental approach.
In conclusion, we have shown that S18986 crosses the BBB intensively and that it mainly accumulates on or in brain hippocampal cells. We conclude that comprehension of bECF/bICF and bECF/CSF partitioning is necessary for understanding the neuropharmacokinetics of a drug and that investigation of bECF to plasma partitioning alone can lead to error, in particular in understanding the transport processes involved at the BBB.

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References

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