Distribution of Gacyclidine Enantiomers in Spinal Cord Extracellular Fluid

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Purpose. Determination of the pharmacokinetics of gacyclidine enantiomers, a non-competitive NMDA antagonist, in plasma and spinal cord extracellular fluid (ECF) of rats.

Methods. Implantation of microdialysis probes in spinal cord (T9). Serial collection of plasma samples and ECF dialysates over 5 hours after IV bolus administration of (±)-gacyclidine (2.5 mg/kg). Plasma protein binding determined *in vivo* by equilibrium dialysis. Chiral GC/MS assay.

Results. Plasma concentrations of (+)-gacyclidine were ~25% higher than those of (−)-gacyclidine over the duration of the experiment in all animals. Plasma concentrations decayed in parallel in a biphasic manner ($t_{1/2}\alpha$ ~9 min; $t_{1/2}\beta$ ~90 min) with no significant difference between enantiomers. Clearance and volume of distribution of (−)-gacyclidine were approximately 20% higher than those of its optical antipode (CL: 248 *vs* 197 $ml.kg^{-1}.min^{-1}$; Vd_β: 31.6 *vs* 23.5 l/kg). Protein binding (~90%) was not stereoselective. Both gacyclidine enantiomers were quantifiable in spinal cord ECF 10 min after drug administration and remained stable over the duration of the experiment in spite of changing blood concentrations. Penetration of (−)-gacyclidine was significantly higher (~40%) than that of (+)-gacyclidine in all animals. Yet, exposure of spinal cord ECF was similar for both enantiomers, and not correlated with plasma AUCs.

Conclusions. The disposition of gacyclidine enantiomers is stereoselective. Both enantiomers exhibit a high affinity for spinal cord tissue and their distribution may involve a stereoselective and active transport system. This hypothesis could also explain the discrepancy between drug concentrations in plasma and spinal cord ECF.

KEY WORDS: enantiomers; extracellular fluid; gacyclidine; microdialysis; spinal cord.

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ABBREVIATIONS: ECF, extracellular fluid; GC-MS, gas chromatography/mass spectrometry; AUC_{Plasma}, area under the total plasma concentration-time curve from time 0 to time 300 min; $AUC_{Plasma\ Free}$, Area under the free plasma concentration-time curve from time; 0 to time 300 min; AUC_{ECF}, area under the extracellular fluid concentration-time curve from time 0 to time 300 min.

INTRODUCTION

One major problem in interpreting *in vivo* data in terms of receptor interactions is the interposition of pharmacokinetic processes that control drug availability in the biophase. This is especially true for centrally acting drugs, due to the presence of cerebral barriers, and for chiral compounds which can exhibit stereoselective pharmacokinetics (1–3). Thus, monitoring free drug concentrations in tissue is crucial to better understand the time course of drug effects, and to optimize drug dosing regimens.

Gacyclidine (*cis* (pip/Me) 1-[1-(2-thienyl)-2-methylcyclohexyl] piperidine), a non competitive *N*-methyl-D-aspartate (NMDA) antagonist, is a chiral drug (Fig.1) with neuroprotective properties (4,5). Proposed in the treatment of central nervous system injuries, its pharmacokinetic properties in the target tissue remain unknown, yet are essential to better understand the time course of its pharmacological effects and should contribute to improve its clinical efficacy.

Consequently, the purpose of this study was to determine the pharmacokinetics of gacyclidine enantiomers in rats by using the traditional approach of plasma data analysis together with the determination of the concentration-time profiles of free gacyclidine enantiomers in spinal cord extracellular fluid (ECF) by microdialysis. This work also highlights the potential value of microdialysis in the study of drug pharmacokinetics at its site of action. Indeed, this approach has not yet been applied to the determination of free interstitial drug levels in this specific tissue.

MATERIALS AND METHODS

Drugs and Chemicals

Gacyclidine (racemic mixture, (+) and (−) enantiomers) and phencyclidine were supplied by Institut Henri Beaufour (Paris, France). All other chemicals were of reagent grade, obtained from commercial suppliers, and used without further purification.

Animals

Male Wistar rats weighing 300–340 g were obtained from Elevage Dépré (Saint Doulchard, France). They were housed in conventional facilities in groups of five per cage and maintained in a controlled environment (20 ± 2°C, 65 ± 15% relative humidity) with a natural light-dark cycle. They were allowed to adapt to the housing environment for at least one week prior to study and had access to food (U.A.R., Villemoisson sur Orge, France) and tap water ad libitum. All animal procedures adhered to the "Principles of laboratory animal care" (NIH publication #85-23, revised 1985).

Anesthesia

Rats (n = 6) were anesthetized with isoflurane (induction: 5% and maintenance: 1–1.5% isoflurane in air) by an Isotec 4 evaporator (Ohmeda, Maurepas, France) and placed onto a heating pad set at 37–37.5 °C (Homeothermic blanket system,

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Phymep, Paris, France). They were mechanically ventilated at 80 cycles/min with a small animal respirator (Harvard Biosciences, Les Ulis, France) over the duration of the experiment. End-tidal CO_2 was monitored with a CO_2 analyzer (Engström Eliza, Sweden) and maintained between 4.0 and 4.7% through manual adjustment of respirator settings. A polyethylene catheter (N°3, Biotrol, Paris, France) filled with heparin-saline solution (25000 UI/l, Héparine Choay, Paris, France) was inserted into the right carotid artery and used for blood sampling. A second catheter (N°1, Biotrol, Paris, France) inserted into the left jugular vein was used for drug administration.

Microdialysis

Spinal Cord Microdialysis

After shaving, a dorsal midline incision was made on the skin of the back at the T8 to T11 level. Paravertebral muscles were detached and adipose tissue separated to expose the dorsal laminae. Laminectomy was performed at a single thoracic level (T9) to expose the corresponding spinal segment, and dura mater opened with a thin injection needle. Before implantation, microdialysis probes (CMA/11, membrane length: 4 mm, cut-off: 6 kDa, O.D.: 240 μm, Carnegie, Phymep, France) were flushed with Ringer's solution at 15 μl/min to purge membranes and tubing of air bubbles. The flow rate was then reduced to 5 μl/min, the probes inserted into the spinal cord at T8, subsequently moved rostrally up to 5–6 mm above the laminectomy, and allowed to equilibrate for 30 min. Finally, probes were checked for the presence of air bubbles at the end of each experiment. *Calibration of Microdialysis Probes*. Relative gacyclidine enantiomer microdialysis probe recovery was estimated by *in vivo* reverse dialysis. In this approach, the substance of interest is introduced into the perfusate and one assumes that its relative loss during the perfusion (delivery) is an estimate of the recovery (6). Recoveries were determined in a dedicated group of rats (n = 4) using the same experimental protocol as described above. After implantation in spinal cord, the probe was perfused with Ringer's solution spiked with gacyclidine (80 ng/ml) at a flow rate of 5 μl/min. Dialysates were serially collected every 20 min over 3 h and frozen (−20°C) until assayed. The mean *in vivo* recovery computed from all recovery ratios calculated as shown below:

$$\text{Recovery}_{\text{in vivo}} = (1 - C_{out} / C_{in}) \times 100$$

where C_{in} and C_{out} are the drug concentrations in the perfusate inflow and outflow, respectively.

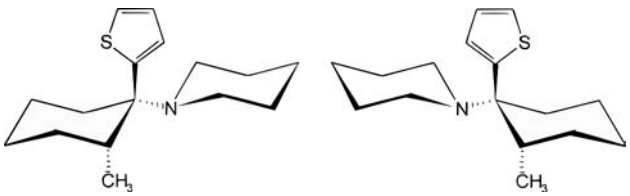

Fig. 1. Chemical structures of gacyclidine enantiomers. Left: (−)-1*S*, 2*R*-gacyclidine; Right: (+)-1*R*, 2*S*-gacyclidine.

Histology

At the end of each experiment, animals were deeply anesthetized. All blood from the circulation was removed by perfusing the heart with saline (200 ml) and severing the inferior vena cava. Subsequently, *in situ* fixation was performed by perfusing 200 ml of 10% neutral buffered formalin, and the spinal column from T7 to T10 removed. The spinal cord was carefully dissected out of the vertebrae and placed in 4% buffered formalin for at least 7 days for tissue fixation. Spinal cord tissue was later embedded in paraffin, and serially cut with a microtome. Every 10^{th} 5 μm section was routinely stained with hematoxylin pholine saffron (HPS) and used for microscopic examination. The whole path of the semipermeable part of the microdialysis membrane and the implantation site could thus be screened for morphological changes.

Pharmacokinetic Studies

Each animal received a single IV bolus dose of racemic gacyclidine (2.5 mg/kg of base) *via* the jugular vein. The catheter was then flushed with 0.2 ml of isotonic saline. Blood (200 μl) was collected through the arterial catheter by means of 1 ml disposable plastic syringes before dosing and at 5, 10, 20, 30, 60, 90, 120, 180, 240 and 300 min after drug administration. After each collection, an equal amount of heparinized saline was injected to flush the catheter and to maintain the fluid volume. Immediately following collection, blood samples were transferred into 1.5 ml microcentrifuge tubes (Eppendorf, Polylabo, Strasbourg, France) containing 5 μl of heparin (25 000 IU/l; Héparine Choay) and centrifuged at 5 600 g for 10 min. Dialysates (100 μl) were continuously collected for periods of 20 min over 300 min post-injection by means of a microfraction collector (CMA/140, Carnegie, Phymep, France). Plasma samples and dialysates were kept at −20°C until analysis.

Plasma Protein Binding

Binding of gacyclidine enantiomers was determined by equilibrium dialysis from plasma of individual rats (n = 5) spiked with racemic gacyclidine at concentrations of 20, 50 and 200 ng/ml. Briefly, plasma samples (200 μl) were dialyzed against 0.15 M, pH: 7.4 phosphate buffer (200 μl) at 37°C with constant stirring at 8 rpm during 3 h using an equilibrium dialysis system (Dianorm®, Braun ScienceTec, Les Ulis, France). The fluids in the dialysis cells were separated by Spectrapor® dialysis membranes (m.w. cutoff: 10 kDa; Spectrum Medical Industries, Los Angeles, USA). At the end of dialysis, plasma and buffer samples were collected and frozen at −20°C until analysis. The bound fraction (f_b) of each gacyclidine enantiomer was then calculated according to the general equation:

$$f_b\ (\%) = (1 - \text{Conc}_{\text{buffer}}/\text{Conc}_{\text{plasma}}) \times 100$$

Non-specific drug adsorption onto the dialysis membrane was determined for each concentration in triplicate to provide a correction factor taken into account, when appropriate, in the calculation of the bound fraction.

Finally, the average plasma protein binding was used to calculate the concentrations of free gacyclidine enantiomers in plasma from total concentrations.

Drug Analysis

Enantioselective Assay

Concentrations of gacyclidine enantiomers in plasma and dialysates were determined by an enantioselective GC-MS assay (7). Briefly, to 100 μl of dialysate or 100 μl of plasma was added 10 μl of internal standard solution (phencyclidine, 0.2 μg/ml in methanol). After alcalinisation to pH 8.0 by adding 10 μl of Tris buffer, the mixture was extracted with 3 ml of hexane on a vortex-mixer for 1 min. After centrifugation (4 min at 3000 g), the organic upper layer was transferred into a conical glass tube and evaporated to dryness at 35°C under a stream of nitrogen gas. The residue was dissolved in 50 μl of methanol and an aliquot (2 μl) injected into the GC-MS system. A GC 8000 gas chromatograph equipped with a A200 S automatic sampler (Fisons Instruments, Arcueil, France) was used. Sample injections were performed in splitless mode. Separation was carried out on a chiral fused-silica capillary column (25 m × 0.25 mm I.D., 0.25 μm film thickness) with a CP-chirasil-Dex stationary phase (Chrompack, Les Ulis, France). Detection was performed by a MD 800 mass selective detector (Fisons Instruments) in electron impact ionization mode (70 eV ionization energy). Chromatograms were generated in selected-ion monitoring mode. Gacyclidine enantiomers were quantified by detecting the total ion current of m/z 206 for gacyclidine, and of m/z 200 for phencyclidine (internal standard). Enantiomers of gacyclidine were identified according to predetermined retention times (27.9, 30.1 and 31.5 min for phencyclidine, (+)-gacyclidine and (−)-gacyclidine, respectively). Calibration curves of gacyclidine enantiomers (1.5 to 200 ng/ml and 1.5 to 100 ng/ml, respectively) were prepared by spiking rat plasma and Ringer's solution, and extracted as described above. The inter-assay coefficient of variation over the whole concentration range was between 1 and 14% (n = 10), and the intra-assay coefficient of variation ranged from 3 to 15% (n = 6). In plasma and dialysates, the limit of detection (signal to noise ratio of 3) was 0.5 ng/ml and the limit of quantitation was 1.5 ng/ml for each enantiomer. The extraction efficiency of the enantiomers from plasma and Ringer's solution was higher than 90%.

Non-Enantioselective Assay

Briefly, separation was achieved by GC-MS on a Chrompack CPSil-8CB capillary column (25 m × 0.25 mm I.D., 0.12 μm film thickness). Helium was used as a carrier gas at a column head pressure of 100 kPa. The thermal program consisted in an initial hold at 60°C for 1 min, increased to 190°C at a rate of 25°C/min, then reached a final temperature of 200°C at 5°C/min. The injection port was held at 210°C and was operated in the splitless mode. The run time was 9 min. The mass spectral measurements and the extraction procedure were carried out in the same conditions than those described for the enantioselective assay. The extraction efficiency was close to 100%. The calibration curves were linear over the range of 1–200 ng/ml. The inter- and intra-assay coefficient of variations over the whole concentration range were less than 12% (n = 10) and the limit of quantitation was 1 ng/ml. This method allowed us to check that the sum of the plasma concentrations of the two enantiomers measured by the enantioselective assay was similar to the plasma concentrations of racemic gacyclidine determined by the non-enantioselective assay from a pooled rat plasma collected in a dedicated group of healthy animals (n = 5) who received racemic gacyclidine (2.5 mg/kg i.v.).

Pharmacokinetic Analysis

The pharmacokinetic parameters of gacyclidine (racemic mixture and individual enantiomers) were determined for each rat using standard non compartmental analysis techniques. In addition, plasma concentration-time curves were fitted to a two-compartment open model by non-linear least-squares regression (MicroPharm, version 5.0, LogInserm, Paris, France). The choice of the model was based on the Akaike information criterion. Rate constants (α, β), mean residence time (MRT), half-lives ($t_{1/2}\alpha$, $t_{1/2}\beta$), clearance (CL), and volumes of distribution (V_c, Vd_{ss}, and Vd_β) were calculated with standard pharmacokinetic equations (8). Areas under the concentration-time curve (AUC) were calculated by the trapezoidal rule from time 0 to 300 min (AUC_{0-300}) in plasma and ECF for comparison purposes, and from time 0 to infinity (AUC_∞) in plasma.

Gacyclidine concentrations in dialysates (C_D) were time-averaged over the collection interval, and corrected by the *in vivo* recovery (R) to yield extracellular concentrations:

$$\text{Extracellular concentration} = (C_D \times 100)/R$$

The extent of drug transport into spinal cord ECF (gacyclidine penetration) was expressed as the ECF to plasma AUC ratios determined over the duration of the experiment (300 min).

Statistical Analysis

All values are reported as mean ± standard deviation. Statistical comparisons were performed by using the Wilcoxon signed rank test for paired data (Statview version 4.5, Abacus Concepts, Berkeley, USA) with the *a priori* level of significance set at $p < 0.05$. The Pearson product moment correlation coefficient (r) was used to evaluate the strength of the relationship between plasma and spinal cord ECF AUCs.

RESULTS

Histology

Spinal cord tissue reactions to the implantation of the probe were characterized by the presence of local hemorrhage along the path of the probe. However, no relationship between the presence and/or the severity of the hemorrhage and gacyclidine penetration could be found.

Protein Binding

In vitro protein binding was constant over the range of concentration studied. Gacyclidine was highly bound to plasma proteins (f_b: 89.9 ± 2.8% for (+)-gacyclidine vs. 89.3 ± 3.5% for (−)-gacyclidine) with no significant difference between the two enantiomers (n = 15). Adsorption to the dialysis membrane was negligible (<2%). Free gacyclidine concentrations predicted in plasma were in reasonable agreement (Fig. 2) with those estimated *ex vivo* by equilibrium dialysis from a pooled rat plasma collected in a dedicated group of healthy animals (n = 5) who received racemic gacyclidine (2.5 mg/kg i.v.).

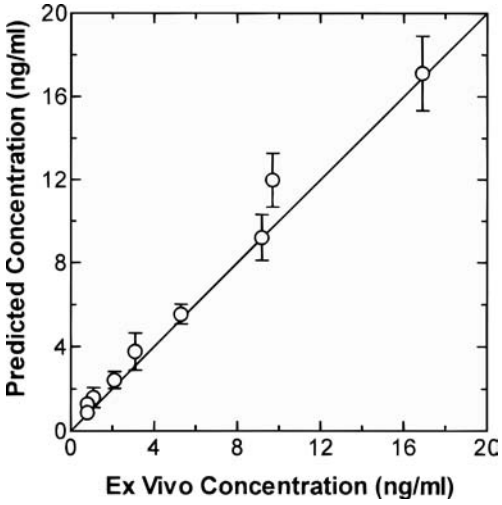

Fig. 2. Correlation between free gacyclidine concentrations predicted from total plasma concentrations and free gacyclidine concentrations measured *ex vivo*. The solid line represents the line of identity.

Gacyclidine Pharmacokinetics

Mean plasma and ECF concentration-time profiles of (+) and (−)-gacyclidine enantiomers are shown in Fig. 3. Plasma concentrations of racemic gacyclidine, determined by summing the concentrations of both enantiomers, were similar to those measured by the non-enantioselective assay. Gacyclidine disposition after i.v. administration was best described by a biexponential equation in all animals. Pharmacokinetic parameters are summarized in Table I. There were no differences in distribution half-life ($t_{1/2}\alpha$), terminal half-life ($t_{1/2}\beta$) and mean residence time (MRT) for the two enantiomers. In contrast, concentrations of (+)-gacyclidine were ~25% higher ($p < 0.01$) than those of its optical antipode over the length of the experiment and in all animals. Accordingly, AUC_{Plasma} of (+)-gacyclidine was ~26% higher than that of (−)-gacyclidine ($p < 0.02$). Both total body clearance (CL) and volumes of distribution (V_C, Vd_{SS}, and Vd_β) of (−)-gacyclidine were significantly higher (ca. 20% increase) than that of (+)-gacyclidine.

Table II lists AUCs in spinal cord ECF, ECF/plasma AUC ratios, and the enantiomeric ratios. *In vivo* recoveries of gacyclidine enantiomers (54.4 ± 10.8% and 53.9 ± 11.0% for (+)- and (−)-gacyclidine, respectively) reached an equilibrium within 20 min after the start of the perfusion. Gacyclidine concentrations in spinal cord ECF reached a maximum within 20 min after drug administration (range: 5.7 to 14.5 ng/ml for (+)-gacyclidine and 5.2 to 15.5 ng/ml for (−)-gacyclidine), then remained constant (average ± SD: 7.8 ± 2.7 ng/ml for (+)-gacyclidine and 8.5 ± 2.9 ng/ml for (−)-gacyclidine) with an enantiomeric ratio close to 1.0 over the duration of the experiment. Gacyclidine exhibited an important affinity for the spinal cord tissue although spinal cord exposure (AUC_{ECF}) was highly variable between animals and not significantly correlated to AUC_{Plasma} ($r = -0.57$; $p > 0.05$). Penetration of (−)-gacyclidine in spinal cord ECF, determined as AUC_{ECF}/AUC_{Plasma}, was significantly higher (40 ± 15%) than that of (+)-gacyclidine in all animals.

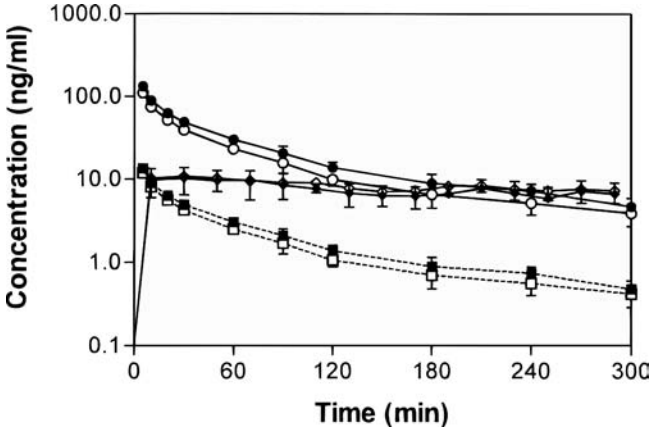

Fig. 3. Concentration-time profiles (mean ± SD; n = 6) of gacyclidine enantiomers in plasma and spinal cord ECF after i.v. bolus administration of 2.5 mg/kg. Total plasma concentrations of (+)-gacyclidine (●) and (−)-gacyclidine (○); Free (+)-gacyclidine (■) and (−)-gacyclidine (□) concentrations predicted from total plasma concentrations; (+)-Gacyclidine (♦) and (−)-gacyclidine (◇) concentrations in spinal cord ECF.

DISCUSSION

The objective of this study was to determine the pharmacokinetics of gacyclidine, a centrally-active drug with neuroprotective properties under investigation in the treatment of spinal cord injuries, in plasma and spinal cord ECF (target tissue) by microdialysis. A particular attention was paid to minimize experimental artifacts. As such, all experiments were performed in rats since this animal species possesses an arterial blood supply to the spinal cord comparable to that described in humans (9). Similarly, isoflurane was selected as anesthetic agent because of its negligible metabolism (<0.2% of the dose) and minimal impact on renal and hepatic functions (10). Impairment of drug metabolism was also reduced by keeping body temperature within physiological values. Gacyclidine being a weakly basic drug (pK_{mcs}: 7.31), end-tidal CO_2 was continuously monitored to avoid anesthesia-related acidosis known to impair the elimination of chemically related compounds (11,12). Laminectomy was performed at T9 in order to avoid the high mortality associated with cervical or upper thoracic lesions (13). In addition, the experimental procedure was designed to reduce tissue damage, by employing one of the smallest probe commercially available, and to allow the determination of drug levels in spinal cord ECF without laminectomy at the dialysis site. An attempt was made to measure the free fraction of gacyclidine enantiomers in blood by microdialysis. However, we had in the end to rely on the traditional approach of plasma data analysis due to the importance of gacyclidine protein binding and the low *in vivo* recovery (<10%) of microdialysis probes for gacyclidine in blood as determined in a preliminary study (unpublished results). Spinal cord ECF concentration-time profiles were determined by using a high perfusion flow rate coupled with long sampling times in order to increase the absolute recovery (i.e. the total amount of drug that is collected within a given time interval). This approach allowed the collection of large enough dialysates as required by the analytical method, yet enabled the sampling interval to be compatible with the establishment of a pharmacokinetic profile. Probe recovery was estimated in a dedicated group of rats. Although this approach

Table I. Pharmacokinetic Parameters of (+)- and (−)-Gacyclidine Enantiomers in Plasma After i.v. Bolus Injection of 2.5 mg/kg of Racemic Gacyclidine to Healthy Anesthetized Rats (n = 6)

	(+)-Gacyclidine	(−)-Gacyclidine	(+)/(−) Enantiomeric ratio
AUC_∞ (ng.min/ml)	6476 ± 940	5158 ± 533*	1.26 ± 0.06
MRT (min)	85.2 ± 7.5	84.4 ± 10.5	1.01 ± 0.04
$t_{1/2}\alpha$ (min)	8.7 ± 3.6	9.9 ± 3.4	0.87 ± 0.14
$t_{1/2}\beta$ (min)	85.0 ± 20.6	91.4 ± 26.9	0.95 ± 0.11
CL (ml/min per kg)	197 ± 30	248 ± 42*	0.80 ± 0.04
V_C (l/kg)	7.1 ± 1.4	8.9 ± 1.5*	0.79 ± 0.07
Vd_{SS} (l/kg)	17.3 ± 1.7	22.0 ± 3.3*	0.79 ± 0.07
Vd_β (l/kg)	23.5 ± 3.0	31.6 ± 6.4*	0.76 ± 0.10

* Significantly different from (±)-gacyclidine ($p < 0.02$; Wilcoxon signed rank test).

only yields an estimate of the recovery, an alternative approach based on its determination in each animal before performing the pharmacokinetic experiment was impossible due to the extremely long half-life of gacyclidine in spinal cord. Indeed, this would have increased the duration of the experiment beyond acceptable limits for a well-controlled anesthesia in our laboratory. Finally, both gacyclidine assays were fully validated using criteria commonly reported in the literature.

Pharmacokinetic profiles of gacyclidine enantiomers in plasma were characterized by higher concentrations of (+)-gacyclidine than those of its optical antipode over the entire duration of the experiment. In light of the relative constancy of the enantiomeric ratio, gacyclidine enantiomer concentrations may be predicted from plasma levels measured using a non enantioselective analytical assay. The extensive distribution out of the vascular space, as reflected by the high values of the volume of distribution is in accordance with the high lipid solubility of gacyclidine (log P: 7.13). Estimated total body clearances indicate a rapid elimination of both gacyclidine enantiomers. These data are in good agreement with those previously established for chemically related compounds in rats (14–16). Although the terminal half-lives of both enantiomers were similar, the systemic clearance and the volume of distribution of (−)-gacyclidine were significantly higher than that of (+)-gacyclidine. These findings, indicative of stereoselective distribution and elimination processes, contribute to explain the discrepancies observed between the plasma concentrations of individual gacyclidine enantiomers. Since binding to plasma proteins was similar for both enantiomers, the stereoselective distribution of gacyclidine could only be explained by different affinities of enantiomers for tissue proteins. Likewise, the stereoselective elimination of gacyclidine could result from quantitative and/or qualitative metabolic differences between enantiomers. Certainly, one cannot rule out by the present study, whether the difference in the disposition of gacyclidine enantiomers is due to one or both of these mechanisms.

The influx of gacyclidine enantiomers into spinal cord ECF was extremely fast, allowing the rapid attainment of an equilibrium between spinal cord and blood. The exposure of spinal cord ECF to gacyclidine enantiomers was high, as evidenced by AUC_{ECF} up to 7 times higher (range: 3–7) than $AUC_{Plasma\ Free}$ calculated from predicted concentrations of free gacyclidine enantiomers in plasma (based upon *in vitro* plasma protein binding). It may be noteworthy that gacyclidine is the first drug for which the $AUC_{ECF}/AUC_{Plasma\ Free}$ ratio is more than unity since, to our knowledge, all drugs studied with microdialysis have an $AUC_{ECF}/AUC_{Plasma\ Free}$ ratio at best equal to one (17). The exposure of spinal cord ECF, however, displayed a marked inter-individual variability, and no significant correlation could be found between AUC_{Plasma} and AUC_{ECF}. Although methodological artefacts cannot be ruled out (i.e. estimation of microdialysis probe recovery in a separate group of animals), these results may indicate that plasma data alone cannot be used to predict gacyclidine concentrations in spinal cord ECF. One of the most interesting feature of gacyclidine was the immediate achievement of a pseudo steady-state in ECF which persisted over the entire duration of the experiment in all animals. Indeed, ECF concentration profiles of both gacyclidine enantiomers after i.v. bolus injection look strikingly similar to those one would obtain after a constant i.v. infusion of drug into the spinal cord. If concentrations of gacyclidine achieved in spinal cord ECF are therapeutic, one could obtain an immediate neuroprotective effect and maintain it during a prolonged period of time after a single intravenous injection. These profiles along with AUC_{ECF} values higher than those estimated in plasma suggest that gacyclidine transport into spinal cord is governed by mechanisms other than passive diffusion. Hammarlund-Udenaes *et al.* recently established that, in

Table II. Exposure and Penetration of (+)- and (−)-Gacyclidine Enantiomers into Spinal Cord ECF After i.v. Bolus Injection of 2.5 mg/kg of Racemic Gacyclidine to Healthy Anesthetized Rats (n = 6)

	AUC_{0-300} in ECF (ng·min/ml)			AUC_{0-300} ratio ECF/Plasma total		AUC_{0-300} ratio ECF/Plasma free	
	(+)-Gacyclidine	(−)-Gacyclidine	(+)/(−) Ratio	(+)-Gacyclidine	(−)-Gacyclidine	(+)-Gacyclidine	(−)-Gacyclidine
Mean ± SD	2210 ± 491	2432 ± 640	0.92 ± 0.10	0.39 ± 0.12	0.54 ± 0.18*	3.82 ± 1.20	5.06 ± 1.70*
Range	1625 − 2999	1849 − 3472	0.82 − 1.05	0.28 − 0.55	0.36 − 0.78	2.77 − 5.44	3.37 − 7.29

* Significantly different from (+)-gacyclidine ($p < 0.02$; Wilcoxon signed rank test).

case of an active transport and when blood concentrations are above K_m, brain concentrations rapidly reach constant levels in spite of changing blood concentrations (17). Our data would thus seem to indicate that gacyclidine enantiomers may be actively transported into spinal cord ECF and that a 2.5 mg/kg dose of gacyclidine results in blood concentrations higher than the transport process K_m. Since spinal cord ECF gacyclidine concentrations did not change over the duration of the experiment, one can think that K_m is below the free gacyclidine concentration estimated in plasma at the end of the experiment (i.e. ~900 pg/ml). This hypothesis of an enantioselective active transport system appears to be confirmed by the higher penetration of (−)-gacyclidine into spinal cord ECF as compared to that of (+)-gacyclidine (i.e. AUC_{ECF}/AUC_{Plasma} and $AUC_{ECF}/AUC_{Plasma\ Free}$ ratios). Indeed, since our experimental conditions apparently lead to a saturation of the drug transfer process, the affinity would theoretically become one major factor governing the passage of gacyclidine enantiomers into spinal cord ECF. Consequently, the affinity of (−)-gacyclidine for the carrier protein would be higher than that of its optical antipode and would only affect the rate but not the extent of the penetration of the drug into ECF as previously reported for the active absorption of drugs (1). Investigations are currently in progress to confirm the active penetration of gacyclidine enantiomers into spinal cord ECF by means of repeated injections. Studies with specific inhibitors are also being performed to determine whether the passage of the drug from tissue towards blood is governed by passive diffusion or involves an active transport system.

In conclusion, this study demonstrates that microdialysis coupled with a sensitive and stereoselective analytical method can be successfully applied the determination of concentration profiles of drug enantiomers in spinal cord ECF. Gacyclidine distribution and elimination are stereoselective. Penetration into spinal cord ECF, characterized by a sustained tissue exposure to the drug enantiomers, seems to involve an active and stereoselective transport system which remains to be fully identified. However, differences between pharmacokinetic parameters are small, and as such should have no significant impact on drug effects. These findings emphasize the need to determine free drug levels in the tissue of interest since concentrations in plasma may not always reflect those in the biophase.

REFERENCES

1. E. J. D. Lee and K. M. Williams. Chirality. Clinical pharmacokinetic and Pharmacodynamic considerations. *Clin. Pharmacokinet.***5**:339–345 (1990).
2. G. T. Tucker and M. S. Lennard. Enantiomer specific pharmacokinetics. *Pharmacol. Ther.* **45**:309–329 (1990).
3. J. Caldwell. The importance of stereochemistry in drug action and disposition. *J. Clin. Pharmacol.* **32**:925–929 (1992).
4. G. Lallement, J. C. Mestries, A. Privat, G. Brochier, D. Baubichon, P. Carpentier, J. M. Kamenka, H. Sentenac-Roumanou, M. F. Burckhart, and M. Peoc'h. GK11: Promising additionnal neuroprotective therapy for organophosphate poisoning. *Neurotoxicology.* **18**:851–856 (1997).
5. M. Michaud, H. Warren, M. J. Drian, J. Rambaud, P. Cerruti, J. P. Nicolas, J. Vignon, A. Privat, and J. M. Kamenka. Homochiral structures derived from 1-[1-(2-thienyl) cyclohexyl]piperidine (TCP) are potent non-competitive antagonists of glutamate at NMDA receptor sites. *Eur. J. Med. Chem.* **29**:869–876 (1994).
6. E. C. M. De Lange, M. Danhof, A. G. de Boer, and D. D. Breimer. Methodological considerations of intracerebral microdialysis in pharmacokinetics on drug transport across the blood-brain barrier. *Brain Res.* **25**:27–49 (1997).
7. G. Hoizey, R. Vistelle, D. Lamiable, H. Millart, B. Gourdier, and P. d'Arbigny. Determination of gacyclidine enantiomers in human plasma by gas chromatography-mass spectrometry using selected-ion monitoring. *J. Chromatogr. B.* **704**:167–174 (1997).
8. M. Gibaldi and D. Perrier. Pharmacokinetics; Marcel Dekker: New York, 1982.
9. E. Fernandez, R. Pallini, E. Marchese, and G. Talamonti. Experimental studies on spinal cord injuries in the last fifteen years. *Neurological Res.* **13**:138–159 (1991).
10. E. I. Eger. Isoflurane: a review. *Anesthesiology***55**:559–576 (1981).
11. P. Geneste, J. M. Kamenka, S. N. Ung, P. Herrman, R. Goudal, and G. Trouiller. Détermination conformationnelle de dérivés de la phencyclidine en vue d'une corrélation structure-activité. *Eur. J. Med. Chem.* **14**:301–308 (1979).
12. A. K. Done, R. Aronow, and J. N. Miceli. Pharmacokinetic bases for the treatment of phencyclidine (PCP) intoxication. In E. F. Domino (eds), *PCP (Phencyclidine): Historical and Current Perspectives*, NPP Books, Ann Arbor, MI, 1981, pp. 487–496.
13. D. Martin, J. Schoenen, P. Delree, V. Gilson, B. Rogister, A. Stevenaert, and G. Moonen. Experimental acute traumatic injury of the adult rat spinal cord by a subdural inflatable balloon: methodology, behavorial, analysis, and histopathology. *J. Neurosci. Res.* **32**:539–550 (1992).
14. A. K. Cho, M. Hiramatsu, D. A. Schmitz, T. Nabeshima, and T. Kameyama. Pharmacokinetic and pharmacodynamic properties of some phencyclidine analogs in rats. *Pharmacol. Biochem. Behav.* **39**:947–953 (1991).
15. M. Zorbas, S. M. Owens, L. M. Plunkett, and H. Bui. The pharmacokinetics of [^{3}H]1-(1-(2-thienyl)cyclohexyl)piperidine (TCP) in Sprague-Dawley rats. *Drug Metab. Dispos.* **17**:641–645 (1989).
16. A. L. Misra, R. B. Pontani, and J. G. Bartolomeo. Disposition of [^{3}H]phencyclidine in the rat after single and multiple doses. *Life Sci.* **24**:2501–2508 (1980).
17. M. Hammarlund-Udenaes, L. K. Paalzow, and E. C. M. de Lange. Drug equilibration across the blood-brain barrier—Pharmacokinetic considerations based on the microdialysis method. *Pharm. Res.* **14**:128–134 (1997).