0090-9556/02/3002-173–176$3.00
DRUG METABOLISM AND DISPOSITION
Vol. 30, No. 2
479/961688
DMD 30:173–176, 2002
Printed in U.S.A.

BRAIN PHARMACOKINETICS OF A NONPEPTIDIC CORTICOTROPIN-RELEASING FACTOR RECEPTOR ANTAGONIST

CAROLINE KELLER, ARMIN BRUELISAUER, MICHEL LEMAIRE, AND ALBERT ENZ

Novartis Pharma AG, Basel, Switzerland

(Received June 11, 2001; accepted November 7, 2001)

This paper is available online at http://dmd.aspetjournals.org

ABSTRACT:

Corticotropin-releasing factor (CRF) is known to play an important role in the body response to stress. Butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-ethylamine (CP-154,526) is a CRF_1 antagonist showing anxiolytic activities in rats in behavioral models, suggesting that CP-154,526 crosses the blood-brain barrier. However, there is no direct evidence for this. This study determined the pharmacokinetic profile of CP-154,526 in rats after i.v. and p.o. application. After i.v. bolus, the concentration declined in a biphasic manner, the first half-life being 0.9 h and the terminal one being 51 h. Systemic clearance was 36 ml/min/kg, and the volume of distribution was 105 l/kg. Oral bioavailability reached 27%. To study brain pharmacokinetics, rats were given a single dose of CP-154,526 p.o. or i.v. and sacrificed after different post-treatment times. Plasma, cortex, striatum, hypothalamus, hippocampus, and cerebellum concentrations were measured. After i.v. bolus, maximal brain concentration was reached after 20 min. The hypothalamus displayed significantly lower concentrations compared with the other brain tissues. In the p.o. study, the maximal plasma concentration was reached after 30 min, whereas the maximal brain concentration was observed after 1 h and remained stable until 2 h post-treatment, without significant differences between the brain tissues. The unidirectional brain extraction ratio was 27 and 9% at vascular concentrations of 0.08 and 16 nmol/ml, respectively. These results demonstrate a large brain penetration of CP-154,526 after i.v. and p.o. applications and a comparable distribution among the brain regions, except for the hypothalamus, which displayed lower concentrations after i.v. bolus.

Corticotropin-releasing factor (CRF[1]) has been shown to play an important role in the behavioral and physiological response of the body to stress. In humans, CRF and CRF receptor disorders occur in some psychiatric diseases, such as major depression and anxiety (Owens and Nemeroff, 1991; Chalmers et al., 1996). Intracerebroventricular administration of CRF to rats produces similar effects to those observed in animals exposed to stress and are blocked by α-helical CRF_{9-41}, a peptidic CRF receptor antagonist, suggesting that CRF receptors antagonists may be a useful medication for the treatment of mental illnesses, such as major depression or anxiety disorders (Owens and Nemeroff, 1991; McCarthy et al., 1999). Since peptides have little chance to penetrate the blood-brain barrier, attention has been focused on nonpeptidic compounds like CP-154,526 (Fig. 1), first described by Schulz et al. (1996). They showed that CP-154,526 has a high affinity for the CRF_1 receptors, which are responsible for the neuroendocrine regulation of the pituitary and for the cognitive and sensory functions, but not for the CRF_2 receptors, which regulate the neuroendocrine, autonomic, and behavioral actions of brain CRF (Lehnert et al., 1998). Schulz and coworkers (1996) also demonstrated that CP-154,526 blocks the CRF-induced adrenocorticotropin secretion in rats after s.c. application in a dose-dependent way. The compound also displayed antidepressant-like and anxiolytic effects in different in vivo experiments after i.p. or p.o. application (Lundkvist et al., 1996; Chen et al., 1997; Mansbach et al., 1997); however, Griebel et al. (1998) compared the behavioral profile of CP-154,526 with buspirone and diazepam in different anxiety models and found little anxiolytic activity. On the other hand, the compound attenuated the stress-induced reinstatement of drug seeking in rats (Shaham et al., 1998), and defensive withdrawal behavior of rats was decreased after 10 days of chronic treatment (Arborelius et al., 2000).

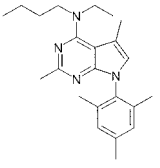

FIG. 1. *Structure of CP-154,526.*

All these studies suggest that CP-154,526 crosses the blood-brain barrier, but there is no direct evidence for this. In this study, the pharmacokinetic profile of CP-154,526 in brain and plasma and its brain uptake index (BUI) were determined by radioactivity measurement and electrospray high-performance liquid chromatography-mass spectrometry (LC/MS).

Materials and Methods

Test Compound. The first part of this study was performed with [^{3}H]CP-154,526 and the second part with nonlabeled substance. The labeling was carried out by the Isotope Laboratories of Novartis Pharma (Basel, Switzer-

[1] Abbreviations used are: CRF, corticotropin-releasing factor; CP-154,526, butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-ethylamine; ,BUI, brain uptake index; LC/MS, liquid chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; AUC, area under the curve; CL, clearance; V_{SS}, volume of distribution.

Address correspondence to: Albert Enz, Novartis Pharma AG, WSJ-386.762, Postfach, CH-4002 Basel, Switerland. E-mail: albert.enz@pharma.novartis.com

TABLE 1

Pharmacokinetic parameters of CP-154,526 after p.o. (13.7 μmol/kg) and i.v. application (2.7 μmol/kg)

Application	Parameter	Unit	Mean ± S.D.
			n = 3
Oral	C_{max}	nmol/ml	0.101 ± 0.035
	T_{max}	h	3.3 ± 1.2
	AUC	nmol/ml · h	1.70 ± 0.19
	Bioavailability	% of dose	27 ± 6
Intravenous	AUC	nmol/ml · h	1.28 ± 0.13
	C_1	nmol/ml	0.303 ± 0.054
	λ_1	l/h	0.803 ± 0.060
	C_2	nmol/ml	0.012 ± 0.002
	λ_2	l/h	0.0146 ± 0.0048
	$t_{1/2,1}$	h	0.87 ± 0.07
	$t_{1/2,2}$	h	51 ± 15
	CL	ml/min/kg	35.9 ± 3.6
	V_0	l/kg	2.55 ± 0.39
	V_{ss}	l/kg	105 ± 30

$t_{1/2,1}$, first half-life; $t_{1/2,2}$, terminal half-life; V_0, volume of initial dilution compartment.

land). Its purity was checked by HPLC. UV and radioactivity evaluation of the chromatograms showed a similar chemical purity as that of the reference standard and a radiochemical purity of >98%. The labeled batch used in this study had a specific radioactivity of 4096 GBq/mmol.

Pharmacokinetics. Oral plasma pharmacokinetic studies were performed with three male Wistar rats (275–310 g; Biological Research Laboratories, Füllinsdorf, Switzerland). The day before dosing, the rats underwent surgical implantation of an indwelling cannula from the right femoral artery to the back of the neck. The animals were individually housed in metabolism cages and were fasted overnight before administrations. The animals were weighed before treatment, and the dosis was calculated for each rat. For the oral dose (13.7 μmol/kg or 800 μCi/kg), [^{3}H]CP-154,526 was dissolved in Placebo G drink solution (Sandoz Ltd., Basel, Switzerland) administered by gastric intubation. For the i.v. dose (2.7 μmol/kg or 1000 μCi/kg), [^{3}H]CP-154,526 was dissolved in a mixture of ethanol (Merck, Darmstadt, Germany)/polyethylenglycol 200 (Merck)/glucose 5% (10:50:40, v/v/v) and administered into the surgically exposed femoral vein in the same animals as those used for the p.o. study after a 1-week interval. Blood samples were taken up to 48 h postdose from the cannulated femoral.

For brain pharmacokinetics, male OFA rats (180–200 g; Biological Research Laboratories, Füllinsdorf, Switzerland) were used. The application dosis was 10 μmol/kg for the i.v. experiment and 30 μmol/kg for the p.o. study. For i.v. dosing, 10 μmol of CP-154,526 was dissolved in 20 μl of 1 N HCl and 1980 μl of saline. Application was made in the tail. For p.o. testing, 30 μmol of compound was solubilized in dimethyl sulfoxide (Merck)/Tween 80 (Merck)/0.9% NaCl (5:5:90, v/v/v). In both cases, this corresponds to an application volume of 2 ml/kg. Plasma and brain samples were collected at times 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 8, and 24 h (four animals per time group) after i.v. application and at times 0.25, 0.5, 1, 2, 4, 8, and 24 h after p.o. administration (three animals per time group). Blood was collected immediately after decapitation, centrifuged for 15 min at 20,000*g* (20°C; Eppendorf centrifuge 5415 C; Hamburg, Germany), and plasma was transferred in Eppendorf tubes. Brains were dissected just after death and frozen on dry ice. All samples were conserved at −70°C until analysis.

BUI. The unidirectional influx for [^{3}H]CP-154,526 was measured by the brain-sampling single-injection technique (Oldendorf, 1970) in adult male Wistar rats (~220 g) under anesthesia [ketamine, 130 mg/kg i.m. (Graeub, Bern, Switzerland); xylazine, 1.3 mg/kg i.m. (Bayer, Leverkusen, Germany)]. A bolus of ~200 μl of either 0.01 M HEPES-buffered Ringer's solution, pH 7.4, or rat plasma was rapidly injected into the common carotid artery. The bolus contained [^{3}H]CP-154,526 at a concentration of 84.9 or 16.4 nmol/ml (9 μCi/ml) together with [^{14}C]butanol (1 μCi/ml; PerkinElmer Life Sciences, Boston MA), the amount of ethanol in the injectant was 0.7 to 1.2% (v/v). The animals were decapitated 5 s after the injection. Samples of the injection solution and the brain hemisphere ipsilateral to the injection side were solubilized in 2 ml of Soluene-350 (Packard, Meriden CT) at room temperature the night before double-isotope liquid scintillation counting. The percentage BUI was calculated as $100 \cdot (^3H/^{14}C\ dpm)_{brain}/(^3H/^{14}C\ dpm)_{injectant}$. The brain extraction ratio E was calculated from $E = BUI \cdot 0.73$, where 0.73 represents the brain extraction ratio of [^{14}C]butanol (Pardridge et al., 1985).

Sample Analysis. The concentration of [^{3}H]CP-154,526 in blood was determined by LC-reversed isotope dilution. The procedure involved the addition of 13.7 nmol of nonradiolabeled CP-154,526 to each blood sample as an internal standard. After adding 1 ml of water, 100 μl of buffer, pH 9 (concentrated 5 times; Merck) and 4 ml of diethyl ether (Merck), the samples were shaken for 30 min and centrifuged (6000*g*, 5 min, 20°C). The supernatant was evaporated in a vacuum centrifuge (Univapo 150H; Zivy, Oberwil, Switzerland). The residue was reconstituted in 250 μl of mobile phase/water and centrifuged (3000*g*, 60 s). The supernatant (200 μl) was analyzed by HPLC (MT2; Kontron Instruments, Zürich, Switzerland) on a Waters Symmetry Shield RP-8 column (5 μm, 150 × 3.9 mm, 60°C; Milford MA) with 0.1% tetramethylammonium hydrogen sulfate/acetonitrile (60:40, v/v; Merck) as mobile phase. The flow rate was 1.2 ml/min; the effluent was monitored at 295 nm. The peak corresponding to the unchanged [^{3}H]CP-154,526 was collected in a polyethylene vial by a fraction collector (SuperFrac; Amersham Biosciences AB, Uppsala, Sweden) and subjected to radioactivity determination. The concentration of [^{14}C]CP-154,526 in each sample was calculated from the ratio of the amount of radioactivity in the eluate fraction corresponding to CP-154,526 and the area of the ultraviolet absorbance of the nonradiolabeled CP-154,526 used as an internal standard. Recoveries averaged 88 ± 6% for blood and 50 ± 10% for brain. The limit of quantification was dependent of the specific radioactivity (i.e., 1.8 and 0.3 pmol/ml after oral and i.v. administration, respectively).

The concentration of CP-154,526 in blood and brain compartments was also determined by LC/MS. Aliquots of plasma (100 μl) were extracted (3 × 15 min on an HS 250 basic IKA shaker) with 500 μl of ethyl acetate (Merck). The combined extracts were then evaporated under a nitrogen stream and dissolved in acetonitrile (Merck) for analysis. The brain samples were homogenized in water (20% brain tissue) using an IKA Ultra-Turrax T8 homogenizer (30 s at position 5; Janke & Kuntel, Staufen, Germany), and aliquots were treated following the same procedure as the plasma samples. For the calibration curves, control biological matrixes (plasma or brain homogenates) were spiked with CP-154,526 (six concentrations) and processed the same way as the samples. Two independent extractions were made for each sample. Quantification of the samples was made by electrospray ionization-LC/MS [HPLC pump, Flux instrument Rheos 4000 equipped with an ERC-3215α degassed and controlled by the Janeiro 1.8e software (Flux Instruments, Basel, Switzerland); MS apparatus, Finnigan Navigator with Masslab 2.0 for Windows NT program (Spectronex, Basel, Switzerland)]. The chromatographic separation was performed on a Macherey-Nagel Nucleosil 100-5 C_{18} (125 × 2-mm i.d.; Oesingen, Switzerland) analytical column, using acetonitrile/water (+ 0.5% formic acid) (7:3) as eluent at a flow rate of 500 μl/min. The MS conditions were as follow: positive ion mode; gas, nitrogen; capillary, 3.5 kV; cone, 35 V; skimmer, 1.5 V; skimmer lens offset, 5 V; hexapole transfect lens, 0 V; source heater, 150°C; low mass resolution, 12.5; high mass resolution, 12.5; ion energy, 0 V; ion energy ramp, 0 V; multiplier, 650 V. Detection was made by selected ion recording at *m/z* 365.5, and quantification of the samples was performed using the Masslab software facilities. The detection limit was 20 fmol/injection.

Data Analysis. The peeling method was applied to describe the data by a biexponential model characterized by $C = C_1 \cdot e^{-\lambda_1 t} + C_2 \cdot e^{-\lambda_2 t}$. The initial estimates of C_1, λ_1, C_2, λ_2 were taken to generate the best fit using the computer software ELSFIT (Sheiner, 1981). The half-lives were calculated as $t_{1/2i} = \ln 2/\lambda_i$. Areas under the curve (AUC) and areas under the first-moment curve were calculated by the trapezoidal rule and extrapolated to infinite time. Total clearance (CL) was calculated as dose/AUC_{iv}. The volume of distribution at steady state was calculated as $V_{SS} = MRT \cdot CL$, where MRT is the mean residence time, calculated as areas under the first-moment curve/AUC.

Results

Based on the dose-normalized AUC ratios, an average bioavailability of 27 ± 6% was estimated. CP-154,526 displayed a large volume of distribution (V_{SS} = 105 l/kg), and a systemic CL of 36 ml/min/kg. After i.v. bolus, the concentration of CP-154,526 in blood declined

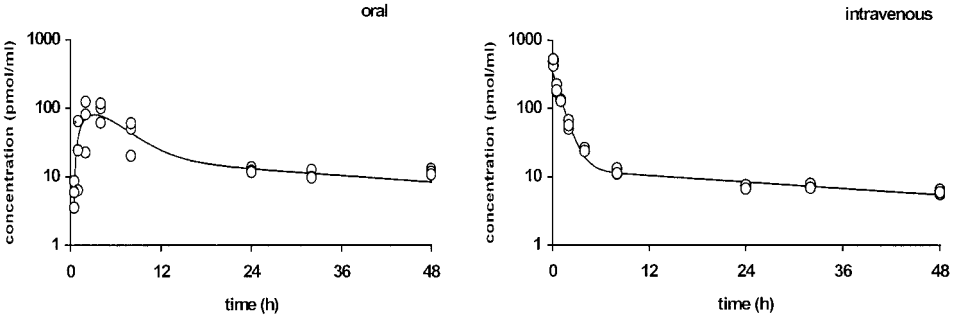

FIG. 2. *Blood CP-154,526 concentrations after oral (13.7 μmol/kg) and intravenous (2.7 μmol/kg) administration (individual values and mean-fitted line).*

TABLE 2

Concentration of CP-154,526 in brain tissues after i.v. application of 10 μmol/kg (nmol/ml or g tissue, mean ± S.D.; n = 3–5)

Time	Plasma	Cortex	Striatum	Cerebellum	Hippocampus	Hypothalamus
0.08	3.51 ± 0.38	1.60 ± 0.23	1.16 ± 0.26	2.46 ± 0.45[a]	1.41 ± 0.42	0.86 ± 0.34
0.17	1.96 ± 0.30	1.29 ± 0.04	1.07 ± 0.12	1.82 ± 0.24[a]	1.18 ± 0.08	0.95 ± 0.24
0.33	1.38 ± 0.28	1.68 ± 0.26	1.25 ± 0.21	2.29 ± 0.56[a]	1.39 ± 0.36	1.09 ± 0.17[a]
0.50	0.99 ± 0.28	1.37 ± 0.33	0.90 ± 0.21	1.53 ± 0.48	1.03 ± 0.30	0.75 ± 0.36[a]
1	0.37 ± 0.05	0.91 ± 0.17	0.63 ± 0.19	0.89 ± 0.17	0.72 ± 0.09	0.29 ± 0.10[a]
2	0.16 ± 0.04	0.35 ± 0.08	0.38 ± 0.08	0.37 ± 0.07	0.36 ± 0.09	0.16 ± 0.13[a]
4	0.05 ± 0.03	0.09 ± 0.04	0.05 ± 0.04	0.10 ± 0.03	0.08 ± 0.04	0.04 ± 0.01
AUC	1812 ± 346	2393 ± 459	1877 ± 457	2742 ± 606	2060 ± 548	1154 ± 505

[a] $p < 0.05$ concentration significantly different from the other brain tissues.

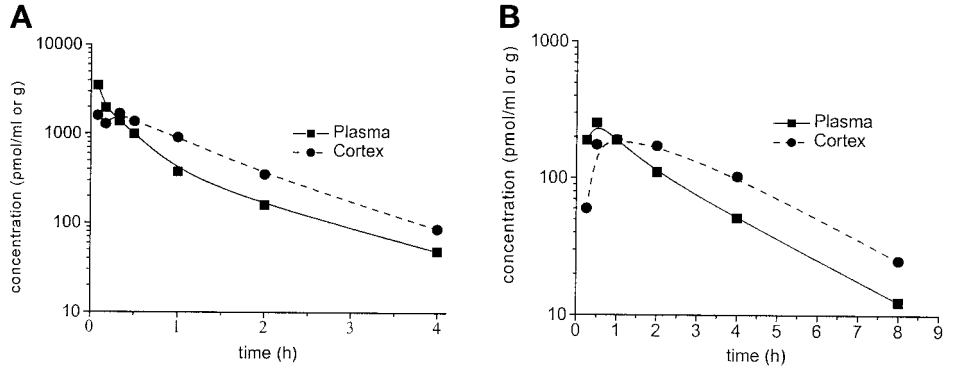

FIG. 3. *A, the CP-154,526 concentration in plasma and cortex after i.v. injection (10 μmol/kg); B, the CP-154,526 concentration in plasma and cortex after p.o. application (30 μmol/kg).*

biphasically with a first half-life of 0.9 h and a terminal half-life of 51 h (Table 1; Fig. 2).

The unidirectional brain extraction of [^{3}H]CP-154,526 was 27 ± 6 and 9 ± 2% after the intracarotid injection of Ringer's buffer containing 0.08 or 16.4 nmol/ml of the compound, respectively. At both concentrations, the brain extraction was strongly decreased when the compound was dissolved in rat plasma to the low, but significant, values of 3 to 4%.

Brain pharmacokinetic was studied by comparing plasma levels of CP-154,526 with the concentration in five brain tissues, namely the cortex, hypothalamus, hippocampus, striatum, and cerebellum. After i.v. application, brain maximal concentrations were reached after ca. 20 min, and no significant differences were observed between the five studied brain tissues, except for the hypothalamus, which exhibited slightly lower concentrations, and for the cerebellum, which showed higher concentrations until 20 min post-treatment (Table 2; Fig. 3A). After i.v. application, the concentration ratio of brain part/plasma reached the maximum of about 2.5 after 1 to 2 h and then slowly decreased in all brain tissues, except again for hypothalamus, where the ratio never exceeded 1 (Fig. 4).

After p.o. application, maximal plasma concentration was reached after ca. 30 min. Brain concentrations increased rapidly to reach maximal concentration after about 1 h and remained stable until 2 h post-treatment (Fig. 3B). No significant differences could be observed between the brain regions (Table 3). No differences in the ratios of brain tissues/plasma were noted between the brain tissues after p.o. application.

Discussion

CP-154,526 displayed a high tissue distribution, revealed by the large volume of distribution (105 l/kg). In accordance with this, an extensive brain distribution with high brain/blood concentrations ratios in both i.v. and p.o. applications could be demonstrated. The brain extraction ratios (9 to 27%) indicate a large and saturable brain penetration of CP-154,526, and the decrease of these ratios to 3 to 4% when using rat plasma as vehicle indicates high plasma protein binding. Since a fairly high systemic clearance (CL = 36 ml/min/kg) is observed, the slow elimination ($t_{1/2}$ = 51 h) may be attributed to the extensive tissue distribution.

CP-154,526 showed good penetration in the different brain tissues, with a maximum already reached after 20 min in the i.v. study. The brain/plasma ratios went up to 2.5 within 1 to 2 h and then slowly

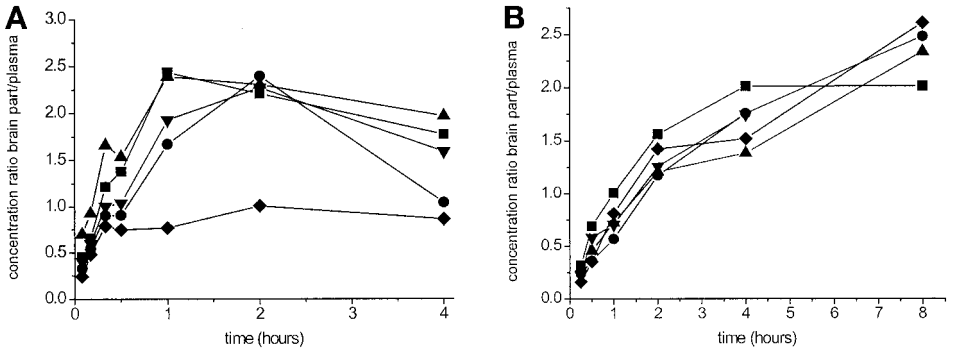

FIG. 4. *The concentration ratios of brain part/plasma of CP-154,526 after i.v. (10 μmol/kg) (A) and p.o. (30 μmol/kg) (B) application.*

■, cortex; ●, striatum; ▲, cerebellum; ▼, hippocampus; ♦, hypothalamus.

TABLE 3

Concentration of CP-154,526 in brain tissues after p.o. application of 30 μmol/kg (nmol/ml or g tissue, mean ± S.D.; n = 3–5)

Time	Plasma	Cortex	Striatum	Cerebellum	Hippocampus	Hypothalamus
0.25	0.19 ± 0.08	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
0.50	0.25 ± 0.02	0.18 ± 0.13	0.09 ± 0.06	0.12 ± 0.09	0.15 ± 0.04	0.09 ± 0.10
1.00	0.19 ± 0.13	0.19 ± 0.10	0.11 ± 0.03	0.14 ± 0.10	0.13 ± 0.09	0.15 ± 0.09
2.00	0.11 ± 0.05	0.17 ± 0.05	0.13 ± 0.03	0.13 ± 0.06	0.14 ± 0.07	0.16 ± 0.05
4.00	0.05 ± 0.03	0.10 ± 0.02	0.09 ± 0.01	0.07 ± 0.02	0.09 ± 0.03	0.08 ± 0.02
8.00	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	BDL	0.03 ± 0.01
AUC	626 ± 155	838 ± 24	650 ± 45	628 ± 17	640 ± 35	688 ± 19

BDL, below detection limit.

decreased, except in the hypothalamus, where the ratio never exceeded 1 and remained stable at this level until 4 h post-treatment. Several hypotheses can be formulated to explain this phenomenon. The blood perfusion is known to be different between the brain regions and, therefore, could influence the penetration of the substance into the brain. Since the transport system is saturable, it can also be supposed that saturation occurred faster in the hypothalamus than in the other brain tissues. Myelin is also distributed differently within the brain and, therefore, increases the lipophilia of the brain regions where it is present in higher concentrations. This means that a lipophilic substance will accumulate more and stay longer in the tissues with high amounts of myelin.

The rat hypothalamus is known to have a lower density of CRF_1 receptors in comparison with other brain tissues, like the brain cortex, whereas the cerebellar cortex displays a higher density of CRF_1 receptors (Chalmers et al., 1995, 1996). It is not clear whether the concentrations of CP-154,526 are a reflection of the receptor's densities, but since CP-154,526 is a highly specific CRF_1 receptor antagonist (Schulz et al., 1996), the possible consequences of the lower hypothalamus and higher cerebellum concentrations of CP-154,526 on its pharmacological activity have to be examined.

In the p.o. study, the concentration ratios still increased 8 h after application (Fig. 4), but no significant differences within the brain regions were observed. Differences between brain tissues seen only in the i.v. trial, where the concentrations are about 10-fold higher than in the p.o. experiment, suggest that a saturation occurs at high concentrations.

In summary, CP-154,526 has been proven to cross the blood-brain barrier in a saturable way well. Correlative studies between behavioral and brain concentrations should be undertaken to determine the minimal brain concentration required for activity, especially for p.o. studies, where the oral bioavailability of 27% can lead to subtherapeutic concentrations in the brain if not taken into account.

Acknowlegments. We thank Pierrette Guntz, Stefan Lehmann, and Dieter Lötscher for technical assistance.

References

Arborelius L, Skelton KH, Thrivikraman KV, Plotsky PM, Schulz DW, and Owens MJ (2000) Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist CP-154,526: behavioral, endocrine and neurochemical effects in the rat. *J Pharmacol Exp Ther* **294:**588–597.

Chalmers DT, Lovenberg TW, and De Souza EB (1995) Localization of novel corticotropin-releasing factor receptor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF_1 receptor mRNA expression. *J Neurosci* **15:**6340–6350.

Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, and De Souza EB (1996) Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* **17:**166–172.

Chen YL, Mansbach RS, Winter SM, Brooks E, Collins J, Corman ML, Dunaiskis AR, Faraci WS, Gallaschun RJ, Schmidt A, and Schulz DW (1997) Synthesis and oral efficacy of a 4-(butylethylamino)pyrrolo[2,3-*d*]pyrimidine: a centrally active corticotropin-releasing $factor_1$ receptor antagonist. *J Med Chem* **40:**1749–1754.

Griebel G, Perrault G, and Sanger DJ (1998) Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* **138:**55–66.

Lehnert H, Schulz C, and Dieterich K (1998) Physiological and neurochemical aspects of corticotropin-releasing factor actions in the brain: the role of the locus coeruleus. *Neurochem Res* **23:**1039–1052.

Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, and Moreau JL (1996) A non peptidic corticotropin-releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol* **309:**195–200.

Mansbach RS, Brooks EN, and Chen YL (1997) Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor antagonist. *Eur J Pharmacol* **323:**21–26.

McCarthy JR, Heinrichs SC, and Grigoriadis DE (1999) Recent advances with the CRF_1 receptor: design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr Pharm Design* **5:**289–315.

Oldendorf WH (1970) Measurement of brain uptake of radiolabeled substances using tritiated water internal standard. *Brain Res* **24:**372–376.

Owens MJ and Nemeroff CB (1991) Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* **43:**425–473.

Pardridge WM, Eisenberg J, and Cefalu WT (1985) Absence of albumin receptor on brain capillaries in vivo or in vitro. *Am J Physiol* **249:**E264–E267.

Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, et al. (1996) CP-154,526: a potent and selective nonpeptide antagonist of corticotropin-releasing factor receptors. *Proc Natl Acad Sci USA* **93:**10477–10482.

Shaham Y, Erb S, Leung S, Buczek Y, and Stewart J (1998) CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing $factor_1$ receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* **137:**184–190.

Sheiner L (1981) ELSFIT. A program for the extended least-squares fit to individual pharmacokinetic data, in *Users Manual February 1981*, Division of Clinical Pharmacology, University of California, San Francisco, CA.