Stereoselective Transport of Baclofen Across the Blood–Brain Barrier in Rats as Determined by the Unit Impulse Response Methodology

Joost B. M. M. van Bree,[1] Corrie D. Heijligers-Feijen,[1] Albertus G. de Boer,[1] Meindert Danhof,[1] and Douwe D. Breimer[1]

Received June 7, 1990; accepted August 12, 1990

The blood–brain barrier transport characteristics of racemic baclofen and the separate R- and S-enantiomers have been determined *in vivo* in rats by using the unit impulse response methodology. Transport rate was determined as blood–brain barrier clearance, the volume of plasma per unit time cleared of baclofen by transport across the blood–brain barrier. Plasma elimination kinetics and CSF elimination kinetics did not differ among racemic baclofen and the R- and S-enantiomers. Transport of each compound could be described by a linear $V(t)$ curve, suggesting the absence of saturable transport processes in the concentration range studied. However, for *R*-baclofen the blood–brain barrier clearance (4.7 ± 1.0 μl/min, mean ± SE; $n = 6$) and cumulative transported amount (0.085 ± 0.007%; $n = 6$) were significantly higher than these values for the S-enantiomer (1.1 ± 0.3 μl/min, 0.031 ± 0.005%; $n = 6$) and racemic baclofen (1.0 ± 0.1 μl/min, 0.036 ± 0.003%; $n = 6$). These findings indicate that there is stereoselective transport of baclofen across the blood–brain barrier.

KEY WORDS: baclofen; blood–brain barrier transport; enantiomers; transport; stereoselective.

INTRODUCTION

Baclofen [4-amino-3-(*p*-chlorophenyl)-butyric acid; Lioresal], which is commonly used as a muscle relaxant, has its primary site of action in the central nervous system (1,2). Since baclofen shows pharmacological activity on peripheral administration, it is assumed that the drug is able to cross the blood–brain barrier (BBB). When taking into account its unfavorable physicochemical properties (zwitter ion at physiological pH and low lipophilicity), it is unlikely that baclofen is transported into the central nervous system (CNS) by passive diffusion alone.

The large neutral amino acid carrier (LNAA) at the BBB is responsible for the transport of circulating amino acids into the CNS (3). Leucine and phenylalanine are the main substrates for the carrier system. Previous studies in an *in vitro* model have shown that baclofen is capable of inhibiting the carrier-mediated transport of leucine across the BBB (4). In addition, using the same *in vitro* model, it has been demonstrated that racemic baclofen is transported by a saturable energy-dependent mechanism (5). It was concluded that, in addition to passive diffusion, baclofen crosses the BBB by means of the LNAA carrier.

The *in vivo* situation is more complex. The LNAA carrier may have different characteristics and the presence of natural substrates or baclofen metabolites may result in competition for the carrier. The aim of the present study was to investigate the transport characteristics of baclofen across the BBB in freely moving rats by means of the recently developed unit impulse response methodology (6). In addition to the racemate, the transport profiles of the active R(−)-enantiomer and the inactive S(+)-enantiomer were determined in order to obtain information about the stereoselectivity of the *in vivo* BBB transport.

METHODOLOGY

BBB transport can be characterized by the time course of cumulatively cleared plasma volume caused by this transport. Extensive theoretical and mathematical considerations have been published elsewhere (6).

Chemicals

Racemic baclofen and its enantiomers and gamma-amino-beta-(2,5-dichlorophenyl)-butyric acid (internal standard) were a gift from Ciba-Geigy, Basle, Switzerland.

Animals and Surgery

Surgical procedures for implantation of cisternal, ventricular, jugular, and carotid canulas have been described previously (6).

Administration Procedure

For icv administration, the cannula of the lateral ventricle was opened and connected to a piece of polyethylene tubing containing a solution of the drug to be studied in saline. A fixed volume of 8 μl was administered instantaneously, with a hand-held syringe (Hamilton, Bonaduz, Switzerland). The total volume of drug solution administered was corrected for the dead volume of the cannula. At regular time intervals, CSF samples were drawn from the cisterna magna cannula and collected in preweighted vials. CSF samples were stored at −20°C until analysis. The objective was to sample for at least three CSF half-lives for each compound. Pilot experiments were performed to determine an adequate icv dose for each compound. The icv doses were 400 ng for the racemate and 400 ng for the R(−)-enantiomer and the S(+)-enantiomer, all calculated as free base. At the end of the experiment the position of the cannula in the lateral ventricle was verified by injection of 5 μl of 0.5% Evans blue solution in saline, then the rat was decapitated, the skull opened, and the brains removed and dissected by a paramedian coronal section. Blue discoloration of the entire ventricular system indicated a correct cannula position.

For iv administration the jugular vein cannula was connected to a motor-driven syringe pump containing a solution of the drug in saline. A fixed volume of 900 μl was infused in 108 sec. The following doses were given: racemate, 2.56 mg; R(−)-enantiomer, 1.5 mg; and S(+)-enantiomer, 2.56 mg.

[1] Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, P.O. Box 9503, NL-2300 RA Leiden, The Netherlands.

0724-8741/91/0200-0259$06.50/0

At regular intervals, CSF and blood samples were obtained. Blood samples of 50 μl were drawn and heparinized. Plasma was obtained by centrifugation. Samples were stored at −20°C until analysis.

HPLC Analysis

Baclofen in Plasma

Ion-exchange columns were prepared by adding 8 g Dowex 50W-2X (Baker, Deventer, The Netherlands) to 80 ml distilled water. Plastic syringes stoppered with glass-fiber plugs were filled with 2 ml of the Dowex suspension. Subsequently, the columns were prewashed with 2 × 0.5 ml water, 2 × 0.5 ml 10% ammonium hydroxide solution, 5 × 0.5 ml water, 2 × 0.5 ml 4 *M* HCl, and 2 × 0.5 ml water. Columns were filled with 0.5 ml water and kept until use.

Before analysis columns were loaded with 0.5 ml 4 *M* HCl and subsequently rinsed with water until the effluent remained neutral. To 25 μl of plasma, 100 μl of the internal standard solution and 600 μl of water were added. Samples were loaded on the Dowex columns, which were washed with 5 × 0.5 ml water. The columns were eluted with 1 ml 10% ammonium hydroxide solution. The eluate was evaporated in vacuum at 60°C. The residue was dissolved in 200 μl water, of which 50 μl was injected into the HPLC system.

The HPLC system consisted of a Spectroflow 400 pump (Spark Holland, Rotterdam, The Netherlands), operating a flow rate of 1.0 ml/min, a WISP 710B autoinjector (Waters Millipore, Etten-Leur, The Netherlands), an Altex 25 cm × 4.5-mm Ultrasphere ODS column (particle size, 5 μm), and a Lambda Max 480 UV detector (Waters Millipore) operated at a wavelength of 220 nm. The mobile phase was composed of 25% acetonitrile and 75% buffer, 0.05 *M* KH_2PO_4 containing 0.005 *M* sodium octanesulfonate, pH 3.0. The linear range of the standard curve employed was 0.4 to 32 μg/ml plasma, the detection limit was 0.2 μg/ml plasma, and the coefficient of variation for the whole curve was smaller than 3.5%.

Baclofen in CSF

Sample size (10–30 μl) was determined by weighing. To the sample, 40 μl internal standard solution was added. Samples were subjected to on-line precolumn derivatization with OPA reagent (50 mg *o*-phthaledialdehyde, 1 ml methanol, 4 ml 0.4 *M* borate buffer, pH 9.2, and 50 μl 2-mercaptoethanol). Immediately before injection, 25 μl of the sample solution was mixed with 40 μl of the OPA reagent. The reaction was allowed to proceed in a fixed-length on-line reaction coil under protection from light. The HPLC consisted of a binary system with Model M45 and Model 501 pumps and an automated gradient controller (Waters Millipore), a PROMIS autoinjector, an Alltech 10-cm Spherisorb ODS-2 column (particle size, 3 μm), and a RF530 fluorimeter (Shimadzu, Tokyo), with an excitation wavelength of 338 nm and an emission wavelength of 465 nm. The mobile phase was composed of methanol/THF (97:3%, v/v) (A) and 70% 0.1 *M* acetate buffer, pH 7.2:30% (B). Initial conditions were 100% B, from 0 to 22 min from 0 to 37% A, from 22 to 26 min from 37% A to 60% A, and from 26 to 28 min from 60% A to 0% A. Between each run, the system was allowed to equilibrate for 15 min in initial conditions. The linear range of the standard curve employed was 40 to 1600 ng/ml CSF, the detection limit was 20 ng/ml CSF, and the coefficient of variation for the whole curve was smaller than 8.8%.

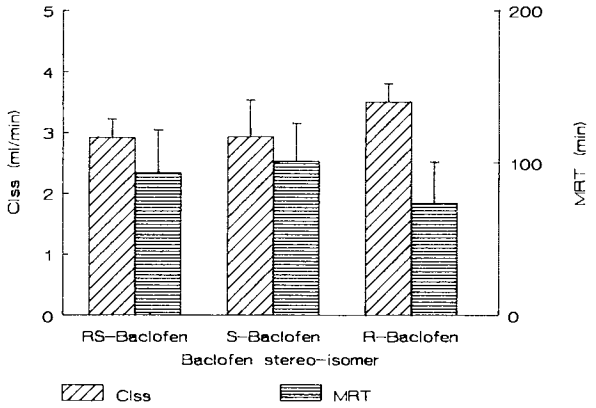

Fig. 1. Plasma kinetic parameters (Clss, clearance in steady state; MRT, mean residence time) of racemic (RS) baclofen and its enantiomers.

Data Analysis

Basic plasma and CSF kinetic parameters were calculated by using model-independent pharmacokinetic analysis (moment analysis). The weighing function was calculated by modeling the CSF data following icv administration to an exponential function with negative exponent, using an extended least-squares optimization procedure (SIPHAR pharmacokinetic modeling software package, SIMED S.A., Creteil, France). The mean weighing function was obtained from the individual constants and exponents. The response function was obtained from CSF concentration–time data after iv dosing. Subsequently, each response function was subjected to the point–area deconvolution with the mean weighing function, and after $I(t)$ was calculated, $V(t)$ was computed. Point–area deconvolution was performed by a custom made APL*PLUS program (STSC Inc., Rockville, MD) on a microcomputer. $V(t)$ was calculated from $I(t)$ and the respective plasma concentration–time curve, using a specially designed spreadsheet application.

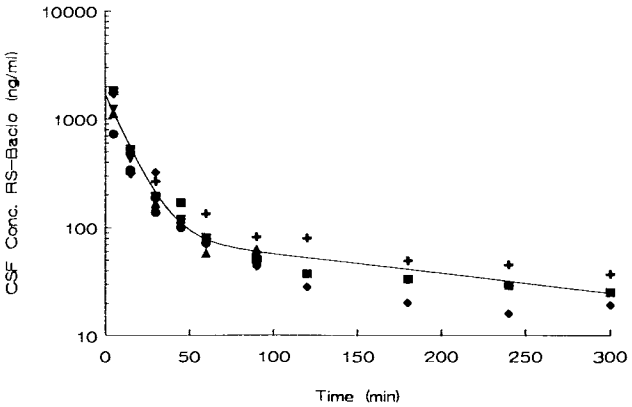

Fig. 2. Unit impulse response of racemic baclofen in CSF after icv administration; each symbol represents a separate animal, and the curve is the response function (n = 6).

Table I. Parameters of the Unit Impulse Response Function $Ae^{-\alpha t} + Be^{-\beta t}$ (Mean ± SE; $n = 6$)

Compound	A (ng/ml)	α (min^{-1})	B (ng/ml)	β (min^{-1})
RS	1635 ± 249	0.083 ± 0.009	85 ± 13	0.0042 ± 0.0009
R	592 ± 172	0.069 ± 0.012	87 ± 10	0.0034 ± 0.0006
S	819 ± 181	0.084 ± 0.011	50 ± 7	0.0017 ± 0.0008

RESULTS

Baclofen concentrations in CSF after icv administration and in CSF and plasma after iv administration could be quantified until 300 min after administration. As depicted in Fig. 1, no difference in plasma clearance [ANOVA $F(2,15) = 3.4$; $P \gg 0.05$] and mean residence time [ANOVA $F(2,15) = 1.9$; $P \gg 0.05$] was found among the racemate and the two enantiomers.

Figure 2 shows the unit impulse response in CSF after administration of the racemate. All unit impulse response functions were described by a polyexponential function. Parameters are given in Table I. CSF elimination parameters for each enantiomer and the racemic mixture are depicted in Fig. 3. No statistically significant differences were found between the enantiomers [CSF elimination clearance—$F(2,15) = 1.5$, $P \gg 0.05$; MRT in CSF—$F(2,15) = 2.6$; $P \gg 0.05$].

An example of the plasma and CSF profile of racemic baclofen after iv administration is depicted in Fig. 4. Deconvolution and subsequent $V(t)$ calculations resulted in the blood–brain barrier clearance, representing the rate of blood to brain transport and the cumulative percentage of material transported into the central nervous system as a measure for the extent of transport. An example of a $V(t)$ curve of racemic baclofen is given in Fig. 5. Correlation coefficients were calculated to evaluate the linearity of the $V(t)$ function. Results are summarized in Table II. One-way analysis of variance showed an overall effect of the compound (enantiomers and racemic baclofen) on both BBB clearance [$F(2,15) = 8.8$; $P \ll 0.01$] and cumulative percentage transported [$F(2,15) = 27.9$; $P \ll 0.001$]. Subsequent level comparison showed that R-baclofen exhibited a significantly higher value for BBB clearance and cumulative percentage transported compared to S- and racemic baclofen. All $V(t)$ curves expressed adequate linearity (Table II). The correlation coefficient range did not change between the different compounds.

DISCUSSION

In the present investigation the blood–brain barrier transport profiles of the separate baclofen enantiomers and of the racemic mixture have been determined by applying the unit impulse response methodology.

Plasma and CSF disposition kinetics did not differ between the different stereo isomers, which is in accordance with findings in dogs (7) and humans (8). Previous *in vitro* experiments with cerebrovascular endothelial monolayers have demonstrated that baclofen transport was concentration and energy dependent, suggesting transport by the carrier system at the blood–brain barrier (4,5).

The present *in vivo* transport profiles exhibited linearity over the concentration range studied and did not show effects of saturable transport. When it is assumed that the *in vitro* and *in vivo* kinetic parameters of the carrier system are comparable, the *in vitro* K_m value of 12 μg/ml (58 μmol) (5) was within the plasma concentration range after iv administration (20–0 μg/ml). Saturation of the carrier system may not have been reached, explaining the linear kinetics observed. Further increase in dose of active enantiomer or racemic baclofen was not possible because severe muscle relaxation would result in respiratory failure. Further, it should be kept in mind that the *in vitro* results were obtained with radioactively labeled racemic baclofen, which resulted in the calculation of "racemic" K_m and V_{max} values. Also, the *in vivo* carrier characteristics may differ significantly from those *in vitro* as caused by the presence *in vivo* of physiological regulation mechanisms which are responsible for "maturation" of carrier characteristics (9). Hence the *in*

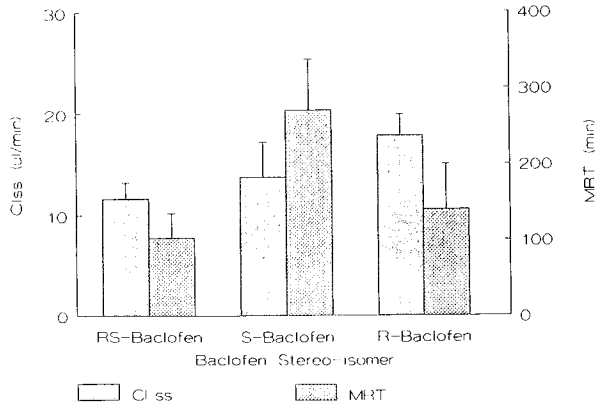

Fig. 3. CSF kinetic parameters obtained from the unit impulse response for racemic baclofen and its enantiomers (mean ± SE; $n = 6$).

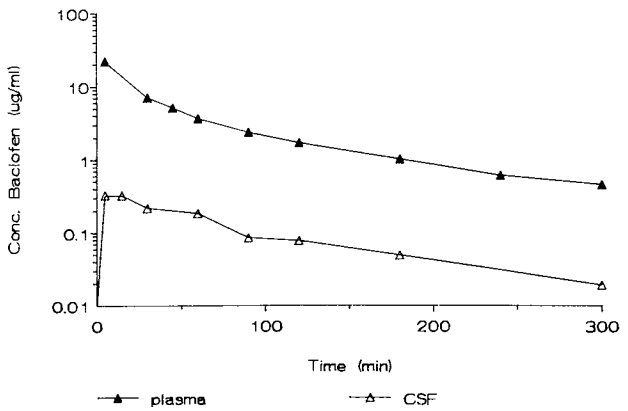

Fig. 4. Example of plasma and corresponding CSF concentration time profile after iv administration of 2.56 mg racemic baclofen.

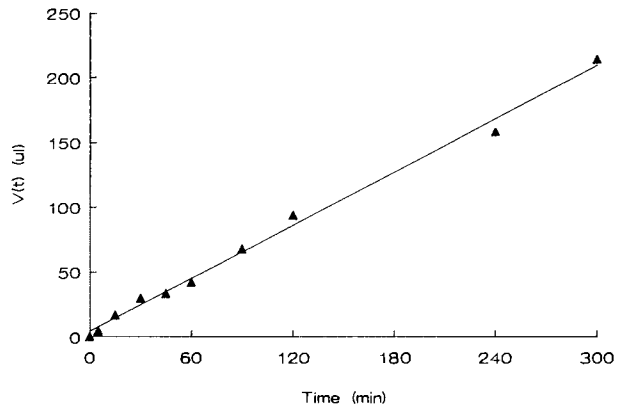

Fig. 5. Example of the cumulative cleared volume-versus-time [$V(t)$] relationship for racemic baclofen in an individual rat.

vitro kinetic characteristics need not necessarily reflect the *in vivo* situation in a quantitative sense.

The present results demonstrate the presence of a stereoselective transport mechanism for baclofen at the BBB. The transport profile of *R*-baclofen differed from those of racemic baclofen and *S*-baclofen with respect to its rate and extent (Table II). In addition, the racemic BBB clearance, as calculated from the mean value between the separately determined values of the consisting enantiomers, is not in accordance with the experimentally obtained value; i.e., passive diffusion is not the only relevant transport mechanism (10). Since plasma protein binding of baclofen is only approximately 30% (in man) (11), stereospecific plasma protein binding cannot explain the current findings. It seems that the R- and S-enantiomers interact with each other at the level of the supposed carrier. It should be noted, however, that the BBB clearance is an overall parameter which reflects the overall process of transport from blood to CSF rather than the characteristics of one particular carrier or transport mechanism. Hence, a detailed mechanistic explanation for the present results would be speculative, since on the basis of these results, we cannot differentiate between potentially explanatory models. When we assume that the diffusion components are comparable, the data suggest that the inactive *S*-baclofen has a higher "overall" affinity for the carrier, while the active *R*-baclofen is transported more efficiently. Hence, the present results suggest that the presence of the S-enantiomer negatively affects the transport of the active R-enantiomer to its site of action.

Future studies using enantioselective bioanalysis will serve to determine the baclofen R/S ratio in plasma and CSF after iv administration of racemic baclofen. On the basis of the current findings, we hypothesize that *S*-baclofen is preferentially transported into the central nervous system.

Table II. Comparison of Blood–Brain Barrier Transport Parameters for Baclofen Racemate and Enantiomers (Mean ± SE; $n = 6$)[a]

Compound	Cl_{BBB} (µl/min)	Amt. transp. (%)	Corr. coeff. range
RS	1.0 ± 0.1	0.036 ± 0.003	0.963–0.999
R	4.7 ± 1.0	0.085 ± 0.007	0.936–0.995
S	1.1 ± 0.3	0.031 ± 0.005	0.956–0.999

[a] Cl_{BBB}, blood–brain barrier clearance; Amt. transp., cumulative amount of baclofen transported into the central nervous system expressed as a percentage of the iv administered dose; Corr. coeff. range, correlation coefficient range of the $V(t)$ curves.

REFERENCES

1. H. U. Fehr and H. J. Bein. Sites of action of a new muscle relaxant (baclofen, Lioresal, Ciba 34 647-Ba). *J. Int. Med. Res.* 2:36–47 (1974).
2. J. W. Faigle, H. Keberle, and P. H. Degen. Chemistry and pharmacokinetics of baclofen. In R. G. Feldman, R. R. Young, and W. P. Koella (eds.), *Spasticity: Disordered Motor Control*, Year Book Medical, Chicago, 1980, pp. 461–475.
3. W. H. Oldendorf. Brain uptake of radiolabeled amino acids amines and hexoses after arterial injection. *Am J Physiol.* 221:1629–1639 (1971).
4. K. L. Audus and R. T. Borchardt. Characteristics of the large neutral amino acid transport system of bovine brain microvessel endothelial cell monolayers. *J. Neurochem.* 47:484–488 (1986).
5. J. B. M. M. Van Bree, K. L. Audus, and R. T. Borchardt. Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharm. Res.* 5:369–371 (1988).
6. J. B. M. M. Van Bree, A. V. Baljet, A. Van Geyt, A. G. De Boer, M. Danhof, and D. D. Breimer. The unit impulse response procedure for the pharmacokinetic evaluation of drug entry into the central nervous system. *J. Pharmacokin. Biopharm.* 17:441–462 (1989).
7. E. W. Wuis, M. J. M. Dirks, E. F. S. Termond, T. B. Vree, and E. Van der Kleijn. Comparison of the pharmacokinetics of intravenously administered rac-baclofen and its (−)-(R)- and (+)-(S)-enantiomers in dogs. *Int. J. Clin. Pharmacol. Res.* 10:239–246 (1989).
8. H. Spahn, D. Krauss, and E. Mutschler. Enantiospecific high-performance liquid chromatographic determination of baclofen and its fluoro analogue in biological material. *Pharm. Res.* 5:107–112 (1988).
9. F. Joo. Current aspects of the development of the blood–brain barrier. *Int. J. Dev. Neurosci.* 5:369–372 (1987).
10. P. Veng-Pedersen. Linear and nonlinear system approaches in pharmacokinetics. I. *J. Pharmacokin. Biopharm.* 16:413–472 (1988).
11. G. J. Mammen (ed.). *Clinical Pharmacokinetics Drug Data Handbook 1989*, ADIS, Auckland, 1989, p. 37.