Transport of a Hydrophilic Compound into the Cerebrospinal Fluid During Experimental Allergic Encephalomyelitis and After Lipopolysaccharide Administration

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Purpose. The transport of the hydrophilic model compound sodium fluorescein into the cerebrospinal fluid (CSF) of rats was studied during experimental allergic encephalomyelitis (EAE), as a model for local central nervous system (CNS) inflammatory disease, and after a single injection of a pyrogenic dose of lipopolysaccharide (LPS), as a model for a general inflammation.

Methods. Transport of sodium fluorescein was measured by means of serial CSF and plasma sampling. Transport of this hydrophilic model compound was studied in Lewis rats suffering from EAA and three hours after LPS administration in male Wistar rats.

Results. During acute EAE, sodium fluorescein concentrations in the CSF increased twofold compared to control animals, whereas plasma kinetics were comparable within both groups. After i.v. LPS administration, however, plasma as well as CSF kinetic parameters of sodium fluorescein concentration were significantly changed from those seen in control animals. Transport of sodium fluorescein from plasma into the CSF was calculated as the ratio Area Under the Curve (AUC) $_{CSF}$ / AUC_{PLASMA}. During acute EAE this ratio increased 2-fold compared to control animals, whereas after i.v. LPS administration it was not significantly different from the one obtained in control animals.

Conclusions. These results suggest an opening of the blood-brain barrier (BBB) during a cerebral inflammatory response, like acute EAE, but not after LPS administration.

KEY WORDS: multiple sclerosis; experimental allergic encephalomyelitis; lipopolysaccharide; blood-brain barrier.

INTRODUCTION

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) that leads to myelin degradation. Experimental allergic encephalomyelitis (EAE) is a experimental autoimmune disease which shares many clinical and histological characteristics with MS and therefore EAE is widely used as an animal model (1). In rats, the clinical signs during EAE may range from atonia of the tail to complete paralysis of the hind limbs, accompanied by a dramatic loss in body weight (2). The pathology of the disease is characterized by the presence of lesions in the CNS, accompanied by perivascular mononuclear cell infiltration and perivascular inflammatory changes (3). There are also clear indications that the integrity of the blood-brain barrier (BBB) is changed during multiple sclerosis (4).

A general inflammatory response can be induced experimentally by injecting animals with the bacterial agent lipopolysaccharide (LPS), extracted from the cell wall of Gram-negative bacteria. An acute phase response develops after LPS administration characterized by fever, general malaise, the release of acute phase proteins, hypotension, suppressed food intake (5) and activation of the hypothalamus-pituitary-adrenal axis (6).

In this present study, changes in transport of sodium fluorescein into the CSF as a marker for the permeability of the BBB during a general inflammation versus a local cerebral inflammatory disease were determined. In order to quantitatively assess the potential differences in transport of the hydrophilic model compound, sodium fluorescein, into the CSF, concentrations were determined after i.v. administration of a pyrogenic dose of LPS and during EAE and were compared to the control situation.

MATERIALS AND METHODS

Materials

Fluorescein-Na ($C_{20}H_{10}O_5Na_2$) and Fluorescein Isothiocyanate Dextran (FD_4 and FD_{20}) are products of Sigma, St. Louis Missouri, USA. Lipopolysaccharide B5;055 (Westphal), Complete Freund's Adjuvants (CFA) and Mycobacterium tuberculosis H37RA were obtained from Difco Laboratories, Detroit, Michigan, USA. All other chemicals were of analytical grade (Baker BV, Deventer, The Netherlands).

Experimental Animals and Surgery

Adult male Wistar SPF rats and adult male Lewis SPF rats (Harlan Sprague Dawley) were obtained from TNO, Zeist, The Netherlands. All animals had a body weight between 185 and 230 g at the day of the experiment. The animals were kept solitary under standard conditions, controlled light and temperature conditions, and food (standard laboratory diets RMH-TH, Hope farms, Woerden, the Netherlands) and water were available *ad libitum*. Acute EAE was induced by a single subcutaneous injection under Hypnorm anaesthesia (0.5 ml/kg bodyweight, i.m.) of 70 µl guinea pig spinal cord homogenate in one hind food pad as described by Matthaei (2). EAE animals were used at day 12 post inoculation.

Two days before the transport experiments, rats received a stainless steel cannula at the cisterna magna under Hypnorm (1 ml/kg bodyweight, i.m.) anaesthesia (7). One day before the transport experiment, polyethylene cannulas were implanted in the left femoral artery and in the right jugular vein under ether anaesthesia.

Experimental Procedure

Transport of sodium fluorescein into the CSF during a general inflammatory response was studied in Wistar rats

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after either saline or LPS administration or in Lewis rats suffering from EAE or control Lewis rats. For the LPS treated animals, at the day of the experiment, 500 µl saline (control) or 500 µl LPS from Escherichia coli B5;055 Westphal, dissolved in saline (2 mg/kg) was administered i.v. to rats via the jugular vein cannula. After three hours, a fixed volume of 500 µl was infused in 1 minute by connecting the jugular vein cannula to a motor-driven syringe pump containing sodium - fluorescein dissolved in saline (20 mg/ml). Every 15 minutes 200 µl blood was collected from the femoral artery cannula, and 10 µl CSF from the cisterna magna cannula was obtained. After saline or LPS administration, colonic temperature was measured every 15 minutes at an ambient temperature of 23 ± 1 °C. Transport of sodium fluorescein into the CSF during EAE was studied similarly and compared to control rats. At the day of the experiment, EAE animals with clinical signs ranging between scale 2 and 3 were used.

Sample and Data Analysis

Sodium fluorescein concentrations were detected by means of HPLC as described by Hurni et al. (8). Plasma samples (100 µl) were extracted with 500 µl acetonitrile (Westburg) and 25 µl 4 M HCl, centrifuged for 10 min at 4000 rpm, and the supernatant was collected and dried at 40 °C in a vortex vacuum evaporator, and the residue was dissolved in 1 ml 0.2 M, pH 10.5. Ten µl FD_4 solution (1.25 µg/ml) as external standard was added and 25 µl (injection volume) was analyzed by HPLC. CSF samples following i.v. administration were analyzed by mixing 10 µl CSF sample with 40 µl FD_4 solution (2 µg/ml or 20 µg/ml) and 100 µl mobile phase. Injection volume was 10 µl for analysis by HPLC.

The concentration-time profiles in plasma and CSF of sodium fluorescein could be individually described by a one compartment model using non-linear extended least square optimization of the modelling package Siphar (SIMED, Créteil, France). The statistical analysis was performed using the S-STAT program of the Siphar pharmacokinetic modelling software. The Fisher test was used for to test the parametric distribution of the data points and the student's t-test was used in order to detect significant differences in pharmacokinetic parameters among the various groups of animals.

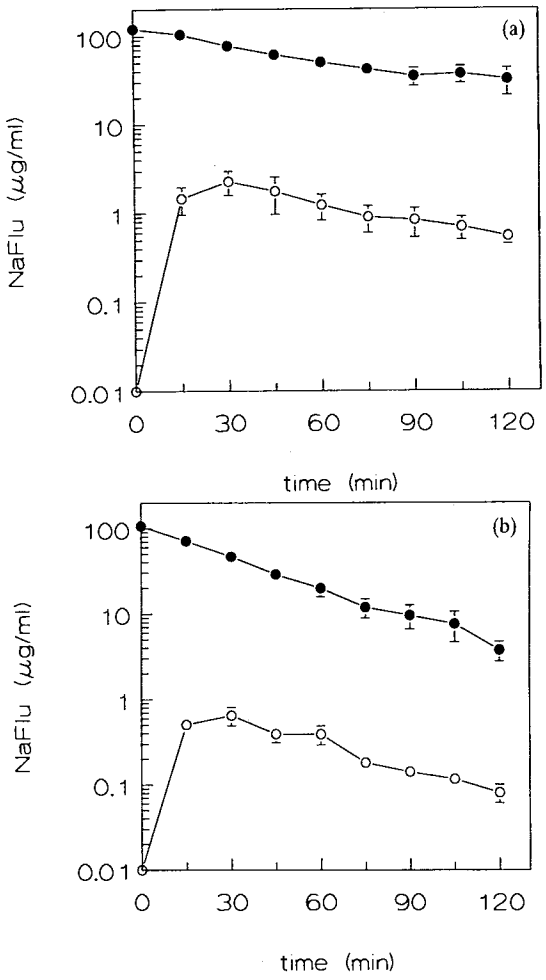

Fig. 1. (a) Plasma- and cerebrospinal fluid concentration-time profiles of sodium fluorescein after i.v. administration of lipopolysaccharide. A pyrogenic dose of lipopolysaccharide (LPS) (2 mg/kg) was administered to Wistar rats. After three hours, sodium fluorescein (NaFlu) was administered and serial plasma- and CSF sampling was performed. By means of HPLC analysis, plasma- (●) and CSF- (○) concentrations were determined. Data are mean ± SEM (n = 8). (b) Plasma- and cerebrospinal fluid concentration-time profiles of sodium fluorescein after i.v. administration of saline (control). Saline was administered to Wistar rats. After three hours, sodium fluorescein (NaFlu) was administered and serial plasma- and CSF sampling was performed. By means of HPLC analysis, plasma- (●) and CSF (○) concentrations were determined. Data are mean ± SEM (n = 7).

RESULTS

A general inflammatory response was induced in rats by i.v. administration of the bacterial endotoxin lipopolysaccharide (LPS). LPS administration to rats resulted in a loss of appetite, changes in behavioral activity, diarrhea, and a hypothermic response within 1½ hrs, followed by a hyperthermic response 3 hrs after LPS administration.

Plasma- and CSF concentration-time profiles of NaFlu in rats 3 hrs after LPS administration are shown in fig 1a, to be compared with the profiles of control animals in fig 1b. Plasma concentrations of NaFlu decreased more slowly ($P < 0.01$) after i.v. LPS administration. Plasma concentration-time profiles of NaFlu in individual rats pretreated with saline or LPS (2 mg/kg) could satisfactorily be described by a one-compartment pharmacokinetic model. The mean pharmacokinetic parameters are shown in table 1.

The plasma kinetics of sodium fluorescein changed significantly after LPS treatment. The area under the plasma concentration curve (AUC) increased 2.3 fold ($P < 0.001$), plasma clearance (CI) values decreased 3-fold ($P < 0.001$), the volume of distribution (V_{SS}) decreased 1.2-fold ($P < 0.05$) and the plasma elimination half-life ($t½_{el,pl}$) increased 2.6-fold ($P < 0.001$) (table 1). The pharmacokinetic parameters of NaFlu in CSF of animals treated with LPS changed significantly. The AUC_{CSF} increased 3.5 fold ($P < 0.05$), and the elimination half-life ($t½_{el,CSF}$) of NaFlu in CSF increased 2.4-fold ($P < 0.05$).

Table I. Pharmacokinetic Parameters of Sodium Fluorescein (50 mg/kg) in Plasma (pl) and Cerebrospinal Fluid (CSF) in Control Animals or in Animals after i.v. LPS Administration. The Pharmacokinetic Parameters (Elimination Half-live ($t_{1/2}$), the Area Under the Concentrations Curve (AUC), and Clearance (Cl) of the Concentration-Time Profiles of Sodium Fluorescein of Rats Administered with Saline or LPS (2 mg/ktg) were Calculated According to a One-Compartment Model. Values are Reported as Mean ± Standard Error of the Mean (SEM). Data were Analyzed Statistically by a Fisher Test, Followed by a Students t-Test

	AUC_{pl} (min · μg/ml)	Cl_{pl} (ml/min)	$t_{1/2,el,pl}$ (min)	AUC_{CSF} (min · μg/ml)	$t_{1/2,CSF}$ (min)	$AUC_{CSF}/AUC_{pl} \cdot (\cdot 10^3\%)$
Control (n = 7)	2556 ± 485	2.7 ± 0.39	27.1 ± 3.95	48 ± 8.7	40 ± 7.6	22.9 ± 5.37
LPS (n = 8)	5946 ± 411[a]	0.9 ± 0.13[a]	62.9 ± 6.99[a]	168 ± 58.9[b]	95 ± 22.6[b]	27.2 ± 8.07

[a] Significantly different from control group ($p < 0.01$).
[b] Significantly different from control group ($p < 0.05$).

In order to compare the percentage of the injected dose transported into the CSF after i.v. administration in LPS treated and control animals, the ratio AUC_{CSF} / AUC_{PLASMA} was calculated (table 1). No significant difference between LPS treated and control animals could be observed (0.023 ± 0.005% and 0.027 ± 0.09%, respectively).

The transport kinetics of NaFlu into the CSF during acute experimental allergic encephalitis (EAE) were studied. Acute EAE was induced in susceptible Lewis rats, and animals developed clinical signs, initiated by weight loss at day 10 post inoculation (p.i.). At day 12 p.i., the day of the transport experiment, all rats had developed full paralysis of the tail and hind limbs. Plasma- and CSF concentration time profiles of NaFlu in animals suffering from EAE are shown in fig 2a to be compared with concentration profiles in control Lewis rats (fig 2b). No significant changes in the plasma kinetic parameters based on the plasma concentration time curve, were observed between control Lewis rats and animals suffering from EAE (table 2). A significant 2-fold increase in the AUC values of NaFlu in the CSF of EAE animals was observed, the AUC_{CSF} values for EAE animals were 121 ± 15.4 min.μg/ml whereas for the control animals AUC_{CSF} values of 57 ± 10 min.μg/ml were found ($P < 0.05$). No significant change in the elimination half-life of NaFlu in CSF between control and EAE animals was detected.

The percentage of the injected dose transported into the CSF was expressed as the ratio AUC_{CSF} / AUC_{PLASMA}. This ratio was 0.022 ± 0.002% in EAE animals whereas this ratio in control animals was 0.012 ± 0.001%, indicating a significant 1.80-fold increase in the amount of NaFlu transported into the CSF during EAE ($P < 0.01$) (table 2).

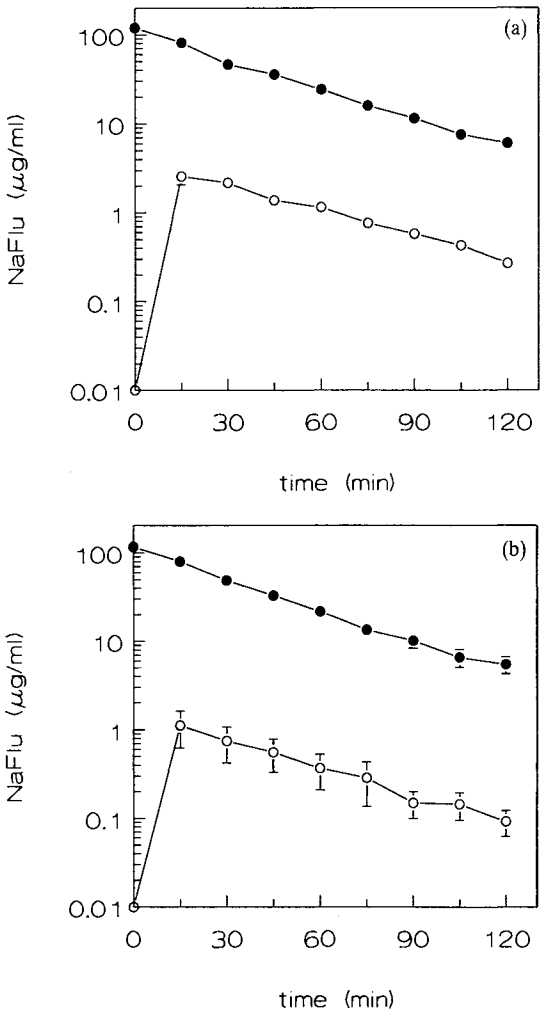

Fig. 2. (a) Plasma- and cerebrospinal fluid concentration-time profiles of sodium fluorescein in Lewis rats with acute EAE at day 12 post inoculation. EAE was induced in susceptible Lewis rats. At day 12 post inoculation, sodium fluorescein (NaFlu) was administered and serial plasma- and CSF sampling was performed. By means of HPLC analysis, plasma- (●) and CSF (○) concentrations were determined. Data are mean ± SEM (n=6). (b) Plasma- and cerebrospinal fluid concentration-time profiles of sodium fluorescein in Lewis rats (control). Sodium fluorescein (NaFlu) was administered and serial plasma- and CSF sampling was performed. By means of HPLC analysis, plasma- (●) and CSF (○) concentrations were determined. Data are mean ± SEM (n=6).

DISCUSSION

The purpose of the present study was to determine alterations in transport of hydrophilic compounds to the central nervous system during experimental allergic encephalitis and after a single injection of a pyrogenic dose of LPS. Since the animals suffered from full-blown EAE on day 12 p.i., maximal opening of the BBB, if occurring, is expected at that time. It was shown that the changes in the transport of compounds into the brain was recovered (to normal level) at day 21 p.i. (9, 10).

A single injection of a pyrogenic dose of the bacterial endotoxin lipopolysaccharide (LPS), was used as a model for

Table II. Pharmacokinetic Parameters of Sodium Fluorescein (50 mg/kg; i.v.) in Plasma (pl) and Cerebrospinal Fluid (CSF) in Control Animals or in EAE Animals at Day 12 Post Inoculation. The Pharmacokinetic Parameters (Elimination Half-Live ($t_{1/2}$), the Area Under the Concentration Curve (AUC), and Clearance (Cl) of the Concentration Time Profiles of Sodium Fluorescein of Control Animals or EAE Animals were Calculated According to a One-Compartment Model. Values are reported as Mean ± Standard Error of the Mean (SEM). Data were Analyzed Statistically by a Fisher Test, Followed by a Students t-Test

	AUC_{pl} (min · μg/ml)	Cl_{pl} (ml/min)	$t_{1/2}$ (min)	AUC_{CSF}	$t_{1/2,CSF}$	AUC_{CSF}/AUC_{pl} ($\cdot 10^3$%)
Control (n = 6)	4593 ± 429	2.3 ± 0.21	27 ± 3.6	57.3 ± 10.01	27.4 ± 3.08	12.5 ± 2.59
EAE (n = 6)	5413 ± 502	1.9 ± 0.18	31 ± 3.5	121.7 ± 15.34[a]	36.5 ± 7.06[a]	22.3 ± 1.26[b]

[a] Significantly different from control group ($p < 0.05$).
[b] Significantly different from control group ($p < 0.01$).

a general inflammatory response. Animals used in this study developed a fever response after a single i.v. injection of LPS characterized by an initial hypothermic response, followed by the hyperthermic response, with a raise in colonic temperature of about 1°C. These characteristics were in accordance with those described by De Rijk and Berkenbosch (11). After i.v. LPS administration, pharmacokinetic parameters of sodium fluorescein in plasma changed dramatically, compared to control rats. A significant increase in plasma AUC of sodium fluorescein between control and LPS treated animals was determined. Systemic administration of LPS to rats caused a substantial decrease in plasma clearance, which resulted in delayed plasma decay of NaFlu after LPS administration. The decrease in plasma clearance of NaFlu after LPS administration is speculated to be the result of an altered glomerular filtration induced by LPS, although further experiments need to be conducted to verify this. Since the driving force for passive transport of sodium fluorescein into the CNS depends on the plasma concentration, the increase in the sodium fluorescein concentration in the CSF of animals treated with LPS is presumably the result of increased plasma concentration rather than an increase in BBB permeability. No significant increase was found in the ratio of AUC_{CSF} over AUC_{PLASMA}, which indicates that the permeability of the BBB is not changed after injection of a pyrogenic dose of LPS.

The fact that no increase or disruption of the BBB could be revealed after i.v. LPS administration in this present study is in accordance with observations of Jeppson *et al.* (12). During sepsis an increase in the brain neutral amino acid content could be observed, which was the result from an increase in the plasma concentration of these amino acids and an activation of the transporter on the cerebral endothelial cells of the BBB. DuMoulin *et al.* (13), however, have described an increase in the permeability of cerebral blood vessels for horse radish peroxidase during sepsis, but these data were not correlated to changes in plasma concentrations of the marker molecule. Since we clearly showed in the present study that plasma concentrations of sodium fluorescein were significantly influenced after LPS treatment, the plasma kinetics have to be taken into account in evaluating increased BBB transport. The integrity of the BBB, however, is changed when LPS is administered via intracisternal inoculation (14).

During EAE, the transport kinetics of sodium fluorescein in the CSF compartment were significantly changed. Plasma kinetics of sodium fluorescein was identical for control and EAE animals. The ratio AUC_{CSF} / AUC_{PLASMA} of NaFlu increased twofold compared to control animals, indicating enhanced transport across the BBB. These observations are in accordance with reports on significant increases in permeability of the BBB in EAE animals for small molecules i.e. radiolabelled sodium and chloride (3,15).

Concluding, during EAE, a significant twofold increase in the transport of sodium fluorescein into the CSF was observed, whereas following a general inflammatory response no changes in transport could be revealed. These observations suggest that cerebral diseases also may have major implications on the transport of compounds, such as therapeutic agents, into the brain. Enhanced permeability of the BBB for endogenous compounds like blood-borne proteins may even contribute to the progression of the disease.

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