MECHANISM OF THE REDUCED ELIMINATION CLEARANCE OF BENZYLPENICILLIN FROM CEREBROSPINAL FLUID IN RATS WITH INTRACISTERNAL ADMINISTRATION OF LIPOPOLYSACCHARIDE

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ABSTRACT:
The mechanism responsible for the reduced clearance of benzylpenicillin (BPC) from the cerebrospinal fluid (CSF) was investigated in rats that received an intracisternal administration of lipopolysaccharide (LPS). BPC was intraventricularly injected and its elimination from the CSF studied. During the inflammation created by the LPS administration to the cisterna magna, the clearance of BPC and taurine from the CSF was significantly reduced but reverted to the control level when N-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor, was intracisternally administered. The in vitro uptake of BPC and taurine was significantly reduced in the choroid plexus (CP, the blood-CSF barrier) of rats with experimental inflammation and in control CP that had been pretreated with sodium nitroprusside (SNP, an NO donor). Interestingly, the clearance and CP uptake of formycin B, a substrate for a nucleoside transporter, were not affected by the experimental inflammation or by pretreatement with SNP. These observations suggest that the BPC transporter, and probably other transport systems as well, is functionally sensitive to NO in the blood-CSF barrier. Therefore, functional impairment of BPC transport in the CP by NO may be partly responsible for the increase in BPC concentration in the CSF during inflammation such as that caused by meningitis.

Meningitis, a potentially fatal disease, is characterized by the inflammation of the meninges that cover the brain and the spinal cords. Secondary to the inflammation, alterations in the function of the barriers between the blood/brain (Wispelwey et al., 1988; Roord et al., 1994; Jaworowicz et al., 1998; Xaio et al., 2001) and blood/CSF, the blood-CSF barrier (Lithander, 1965; Spector and Lorenzo, 1974) have been reported, as evidenced by an increased permeability of normally nonpermeable compounds such as sucrose. In addition to damage to the diffusional barriers, the functional activities of carrier-mediated transports, including penicillins (Lithander, 1965; Spector and Lorenzo, 1974) and glucose (Cooper et al., 1968; Prockop and Fishman, 1968) across the blood-CSF barrier, are reduced with the induction of experimental meningitis. Among these examples, inflammation-dependent change in pharmacokinetics has been studied most extensively for the case of penicillins in the CSF. For example, the intracisternal inoculation of Hemophilus influenzae, has been reported to be associated with a significantly elevated level of ampicillin and BPC in the CSF (Lithander and Lithander, 1966; Spector and Lorenzo, 1974). In addition, a reduction in the transport of BPC was noted in CP (i.e., the blood-CSF barrier) obtained from inflamed rabbits (Spector and Lorenzo, 1974). In general, drug levels in the CSF are partly governed by the kinetics of efflux across the blood-CSF barrier. Therefore, these observations suggest that the transport of BPC from the CSF to the systemic circulation is reduced as the result of the inflammation and that the reduction in the transport is related to the elevation in BPC levels in the CSF of inoculated animals. Despite this implication, however, the underlying mechanism has not been fully elucidated for the inflammation-dependent reduction in transport and elimination. Furthermore, the issue of whether similar mechanisms participate in the reduction in other carrier-mediated transport processes in inflammation of the CSF remains unclear.

NO, a free radical, is endogenously synthesized from L-arginine by NO synthases. Whereas NO itself is relatively reactive, its reaction with superoxide would lead to formation of peroxynitrite, a more chemically reactive specie than NO. These nitrogen oxide forms are known to affect physiological functions via interactions with a variety of DNA, lipids, thiols, aromatic amino acids and transition metals (Geng et al., 1994; Hess et al., 1994; McDonald and Moss, 1994). It has been reported that the biological functions of numerous receptors and enzymes are altered in the presence of these nitrogen oxides (Dimmeler et al., 1994; Kuhn and Arthur, 1996; Mohr et al., 1996). More recently, functional impairment in the transport systems for glutamate, serotonin, and reduced folate in the presence of nitrogen oxides has been reported (Pogun et al., 1994; Trotti et al., 1996; Smith et al., 1999). Therefore, pathological states that involve massive NO production may lead to the functional impairment of important biomolecules such as transporters. Bacterial and viral meningitis activate inducible NO synthase, as evidenced by a significant increase in NO levels in the CSF (Buster et al., 1995). However, the issue of...
whether the pathological level of NO in the CSF is related to a reduction in the functional activity of carrier-mediated transport such as BPC elimination from the CSF is not known.

The objective of this study was to determine the role of excessive levels of NO in relation to the suppression of BPC elimination from the CSF and transport into the CP, the blood-CSF barrier. In addition, to establish the specificity of the NO-mediated effect, CSF clearance and the function of carriers were studied using two model substrates, taurine (Chung et al., 1996) and formycin B (Wu et al., 1993), both of which are eliminated from the CSF by carrier-mediated transport systems, in the presence of pathological levels of NO.

Materials and Methods

The following radioactive compounds were obtained: [3H]BPC (specific activity, 19 Ci/mmol) and [3H]mannitol (specific activity, 57 mCi/mmol) from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK), [3H]taurine (specific activity, 24.1 Ci/mmol) and [3H]formycin B (14 Ci/mmol) from Moravek Biochemicals (Brea, CA). BPC, taurine, LPS (from Escherichia coli), N-nitro-L-arginine, SNP, N-(1-naphthyl)-ethylendiamine dihydrochloride, sulfanilic acid, manganese dioxide and potassium gluconate were purchased from Sigma-Aldrich (St. Louis, MO). Ketamine (Ketalar, Yuhan Co., Seoul, Korea) and acetylcholine (Sedaject; Samu Chemical Co., Chungcheongnam-Do, Korea) were used in this study. All other chemicals were of reagent grade or better and used without further purification.

Animals. Male Sprague-Dawley rats (DaeHan Biolink, Chungbuk, Korea) weighing 270 to 300 g were used in all experiments. Experimental protocols involving animals in this study were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University according to the National Institutes of Health guidelines (National Institutes of Health publication number 85-23, revised 1985) of “Principles of Laboratory Animal Care.”

Estimation of CSF Clearance. The elimination of three model drugs (BPC, taurine, and formycin B) from the CSF was examined in rats by means of a cerebroventricular procedure (Wu et al., 1993; Chung et al., 1996). Rats were anesthetized with ketamine (80 mg/kg) and acetylcholine (10 mg/kg), mounted on a stereotaxic device, and underwent surgery involving the cateterization of the lateral ventricle (LV) and the cisterna magna (CM). When it was necessary to study the kinetics of elimination of BPC, a solution containing unlabeled BPC (volume administered, 5 μl; dose of BPC 1, 50, 200 μg in 5 μl of saline), along with a trace amount of [3H]BPC (17 ng in 5 μl of dosing solution), was injected into the LV cannulae. For the CSF elimination study involving taurine and formycin B, an aliquot (5 μl) of saline containing radio-labeled taurine ([3H]taurine, 5 ng in 5 μl) or formycin B ([3H]formycin B, 6.7 ng in 5 μl) was intraventricularly injected. In all CSF elimination studies, the dosing solution contained [3H]mannitol (i.e., a marker for bulk flow clearance; dose, 76 μg in 5 μl of dosing solution). At preselected time intervals, CSF samples (7 μl) were collected and the radioactivity in an aliquot (5 μl) determined by dual isotope liquid scintillation counting on a Wallac 1414 liquid scintillation counter (PerkinElmer Life Sciences, Turku, Finland). The counting efficiencies for [3H] and [3C] were 40 and 95%, respectively. When necessary, LPS (dose 50 ng) was injected into the CM to induce experimental inflammation in the CSF (Wispelewsky et al., 1988). Wispelewsky and coworkers (1988) reported that the diffusional permeability of the blood brain barrier was found to be the highest after an LPS injection to the rat and, based on the finding, a 4-h pretreatment was used to study the kinetics of BPC, taurine, and formycin B in the CSF in inflammation. In the case of studies of the effect of NO synthase inhibitor administration, N-nitro-L-arginine (dose 0.2 mg in 50 μl dosing solution) was administered via the CM cannulae along with LPS.

Brain Distribution of BPC. The distribution of BPC in selected parts of the brain was examined as described in detail in a previous report (Chung et al., 1996). Briefly, rats underwent stereotaxic surgery as described above. BPC (1 μg as unlabeled BPC; tracer amount, [3H]BPC 17 ng) and [3H]formycin B (76 μg) were administered via the LV cannulae (injection volume, 5 μl). One hour after the administration of the drug, an aliquot of CSF was withdrawn and the rats decapitated. The CP, the olfactory bulb, the cortex, and the cerebellum were then collected, weighed and digested overnight in 3 N NaOH (volume, 100 μl). Subsequently, 3 N HCl (volume, 100 μl) was added to neutralize the mixture and the radioactivity measured by liquid scintillation counting. Inulin, a nonpermeable marker, was used to correct for the amount of extracellular fluid associated with tissue. The distribution of BPC in the brain tissues was expressed as the volume of distribution (Vbrain tissue, viz., tissue to CSF concentration ratio) using the following equation:

\[ V_{\text{brain tissue}} = \frac{dpm [3H]\text{BPC in brain tissue}/g of brain}{dpm [3H]\text{BPC in CSF}/ml of CSF} - \frac{dpm [14C]\text{mannitol in brain tissue}/g of brain}{dpm [14C]\text{mannitol in CSF}/ml of CSF} \]

Uptake of Drugs into Isolated CP. To examine drug transport into the isolated CP in vitro (Chung et al., 1994), rats were decapitated, and the CP isolated from the lateral ventricle. The isolated CP was preincubated at 37°C for 20 min in media containing 250 μM 2,4-dinitrophenol (for ATP depletion), 25 mM HEPES, 40 mM mannitol, and 120 mM KCl (pH 7.4 with Tris). When tissues were treated with NO; SNP (an NO donor, at a final concentration of 1 mM in the preincubation media) was added to the preincubation media. After preincubation, the CP was incubated at 37°C for 5 min (BPC uptake study, Suzuki et al., 1987; formycin B uptake study, Wu et al., 1993) or 20 min (taurine uptake study, Chung et al., 1996) in the appropriate media (media composition for BPC uptake study was 0.1 μM [3H]BPC, 19.5 μM [3H]mannitol, 10 μM unlabeled BPC, 250 μM 2,4-dinitrophenol, 25 mM HEPES, 40 mM mannitol and potassium gluconate; for the taurine uptake study, it was 0.04 μM [3H]taurine, 19.5 μM [14C]mannitol, 25 μM unlabeled taurine, 250 μM 2,4-dinitrophenol, 120 mM NaCl; and for the formycin B uptake study, it was 0.21 μM [3H]formycin B, 19.5 μM [14C]mannitol, 250 μM 2,4-dinitrophenol, 120 mM NaCl). The CP to media concentration ratio (Vcp,rep) representing the transport of drugs into the CP, was calculated as follows:

\[ V_{\text{cp,rep}} = \frac{dpm [3H]\text{substrates in the choroid plexus}/g choroid plexus}{dpm [3H]\text{substrates in media/ml of media}} - \frac{dpm [14C]\text{mannitol in the choroid plexus}/g choroid plexus}{dpm [14C]\text{mannitol in media/ml of media}} \]

Determination of White Blood Cells (WBC) in the CSF. To determine whether the inflammation was induced by the administration of LPS, the numbers of WBC in the CSF were counted before and after the administration. Thus, CSF (10 μl) was collected before and 4 h after injection of LPS via the CM cannulae. Trypan blue (4 mg/ml) was added to an aliquot (5 μl) of the CSF and the number of unstained cells counted using a hemocytometer under a reverse-phase microscope (magnification 10 × 10).

Measurement of Nitrogen Oxides in the CSF. As an indirect index of NO level, the concentration of nitrogen oxides was measured in the CSF. Essentially, nitrogen oxides were converted to inorganic nitrite by a reduction reaction and detected by a photometric method. CSF samples (60 μl) were mixed with reducing reagent (120 μl of 23 mM hydrazinium sulfate, 24 μM copper sulfate, 0.5 M sodium hydroxide). Griess reagent (1% sulfanilic acid in 60 μl of 2 N HCl, 1% N-(1-naphthyl)-ethylendiamine dihydrochloride in H2O, 60 μl) was then added, followed by incubation at 40°C for 5 min for color development, and the absorbance at 550 nm was measured (Green et al., 1982).

Data Analysis. To determine the elimination clearance (CLelimination,CSF) from the CSF and the steady state volume of distribution (Vss) in the CSF for BPC, taurine, or formycin B, the area under the respective CSF concentration versus time curve from time 0 to infinity (AUC0-inf) and the area under the respective first moment time curve from time 0 to infinity (AUMC0-inf) were calculated using standard methods (Gibaldi and Perrier, 1982). Equations 3 and 4 were then used to calculate the clearance and the volume for BPC, taurine, and formycin B, respectively.

\[ CL_{\text{elimination,CSF}} = \frac{\text{Dose}}{AUC_{0-\text{inf}}}, \quad V_{\text{SS}} = \frac{\text{AUMC}_{0-\text{inf}}}{AUC_{0-\text{inf}}} \times CL_{\text{elimination,CSF}} \]
Fig. 1. Temporal profiles of BPC in the CSF after the intraventricular administration of 1-, 50-, and 200-µg dose in control rats.

1. 1-µg dose; ▼, 50-µg dose; ■, 200-µg dose. Each data point represents the mean ± S.D. of at least four separate experiments.

When it was necessary to compare means between the treatments, one-way ANOVA, followed by Duncan’s test, was typically used. For the comparison of BPC distribution in the brain areas, two-way ANOVA, followed by Duncan’s test, was used to compare the treatment (i.e., control versus inflammation) and brain areas (i.e., the CP, the olfactory bulb, the cortex, and the cerebellum).

P < 0.05 was accepted as denoting statistical significance. Data are expressed as the mean ± S.D.

Results

Determination of WBC in the CSF. The WBC count was undetectable in CSF samples prior to LPS administration, but the count became markedly elevated to 2.40 ± 0.432 × 10^7/ml in CSF collected 4 h after the intracisternal administration of LPS (50 ng). In addition, when N-nitro-l-arginine was intracisternally coadministered with LPS, the WBC count was 2.05 ± 0.100 × 10^7/ml, not statistically different from the count obtained with the LPS injection alone. Thus, based on the WBC count, experimental inflammation can be induced by the intracisternal injection of LPS at a dose of 50 ng, and the coadministration of the NO synthase inhibitor did not affect the LPS-induced inflammation.

Measurement of Nitrogen Oxides in the CSF. Based on the photometric assay, the level of nitrogen oxides was 6.86 ± 1.31 µM in the CSF prior to LPS administration. In comparison, the level was increased by 1.73-fold in the CSF obtained 4 h after the intracisternal injection of 50 ng of LPS (13.9 ± 4.59 µM, p < 0.05 from the value obtained prior to the administration, based on the student’s t test), indicating that NO production was markedly enhanced in the CSF.

Elimination of BPC from the CSF. When intraventricularly injected, the temporal profiles for BPC concentration in the CSF exhibited an exponential decline (Fig. 1). In three dose levels of BPC, the bulk flow clearance of CSF, as estimated from intraventricularly administered inulin, was maintained in the range of 5.41 to 8.87 µl/min. No statistical difference was noted for inulin clearance for the three BPC dose levels in control rats. Based on moment analysis, the elimination clearance of BPC from the CSF, at a dose of 1 µg was 21.2 ± 5.34 µl/min (Table 1). As expected, the BPC clearance from the CSF (Table 1) decreased with the dose used (p < 0.05 by one-way ANOVA), indicating that BPC clearance from the CSF is saturable, and probably mediated by a carrier, such as that characterized by Suzuki et al. (1987). The average steady state volume of distribution was in the range of 390 to 588 µl and apparently independent of the BPC dose (Table 1).

When rats were injected with LPS to induce experimental inflammation in the CSF, the elimination clearance of BPC at a dose of 1 µg was 9.53 ± 1.73 µl/min (Fig. 2), indicating that the clearance was reduced to 45% of that in control animals (p < 0.05, one-way ANOVA followed by Duncan’s test). This observation is consistent with findings reported by Spector and Lorenzo (1974). Inulin clearance was 7.14 ± 1.73 µl/min in rats with the experimental inflammation in the CSF, indicating that the bulk flow clearance was not statistically different from that in control rats (i.e., 5.41–8.87 µl/min). When N-nitro-l-arginine, an NO synthase inhibitor, was administered with the LPS, the BPC elimination clearance from the CSF was 21.4 ± 7.18 µl/min (Fig. 2), indicating that the BPC elimination clearance had reverted to the level of the untreated control (21.2 ± 5.34 µl/min; Table 1). Since the administration of N-nitro-l-arginine may nonspecifically increase BPC clearance without any involvement of NO synthase thereby allowing the clearance of BPC to increase to the control level, the CSF clearance of BPC was examined in rats with an intracisternal injection of the NO synthase inhibitor without the coadministration of LPS. The elimination clearance of BPC was found to be 24.8 ± 4.59 µl/min in rats that had been pretreated with N-nitro-l-arginine alone (Fig. 2), indicating that the inhibitor did not nonspecifically increase BPC clearance from the CSF. In addition, N-nitro-l-arginine did not alter the bulk flow clearance (N-nitro-l-arginine alone, 5.63 ± 1.39 µl/min; N-nitro-l-arginine + LPS, 7.78 ± 1.80 µl/min). Collectively, these observations suggest that the reduction in BPC clearance from the CSF may be related to the induction of NO synthase activity in the experimental inflammation of the CSF.

Brain Distribution of BPC. To determine the primary component involved in BPC disposition from the CSF, the distribution of the drug in the brain after an intraventricular injection of BPC was examined. Table 2 shows the Vdbrain tissue, the tissue to CSF concentration ratio for the representative brain area. The concentration of BPC was apparently lower than that in the CSF (i.e., Vdbrain tissue less than unity) and comparable in brain tissues other than the CP. In contrast, the Vdbrain tissue value for the CP was approximately at least 20-fold higher (p < 0.001, two-way ANOVA followed by Duncan’s test) than those in other parts of the brain obtained from either control or inflammation-induced rats. In LPS pretreated rats, the Vdbrain tissue, for CP was statistically different (p < 0.001, two-way ANOVA followed by Duncan’s test) between the control and LPS pretreated rats, indicating that differences in the BPC distribution to the CP contributed to the kinetics of BPC in the CSF. From this observation, the CP appears to be the primary determinant for the kinetics of BPC in the CSF and, as a result, an in vitro examination was further carried out.

BPC Uptake into Isolated CP. BPC uptake into ATP-depleted CP
Control, in control rats; LPS, in LPS administered rats; LPS + l-NA, in LPS administered rats pretreated with N-nitro-l-arginine; control + l-NA, in control rats pretreated with N-nitro-l-arginine. *, statistically different from control by \( p < 0.05 \) by one-way ANOVA followed by Duncan’s test. Each data represents the mean ± S.D. of at least four separate experiments.

**TABLE 2**

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<thead>
<tr>
<th>Brain Region</th>
<th>Tissue to Media Concentration Ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control Rats</td>
</tr>
<tr>
<td>CP</td>
<td>12.8 ± 1.96***</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>0.355 ± 0.0720</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.443 ± 0.219</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.499 ± 0.306</td>
</tr>
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*** \( p < 0.001 \) from other regions of the brain by two-way ANOVA followed by Duncan’s test. ††† \( p < 0.001 \) from control CP by two-way ANOVA followed by Duncan’s test.

was examined in the presence of an outwardly directed \( \text{Cl}^- \) gradient (Suzuki et al., 1987; Ogawa et al., 1994). The uptake of BPC (i.e., \( V_{d,t} \)) was increased with time up to 5 min (Fig. 3, inset), and the incubation time was used to study the BPC transport in the CP. BPC uptake was 3.62 ± 1.22 ml/g in CP obtained from control rats (Fig. 3), whereas this value was 1.30 ± 0.797 ml/g in CP obtained from LPS-administered rats (Fig. 3, reduced by 64% from the control CP, \( p < 0.001 \), one-way ANOVA followed by Duncan’s test). When an uptake study was carried out using control CP pretreated with SNP, an NO donor (Kovaluk et al., 1992), for 20 min, the \( V_{d,t} \) for BPC was 1.21 ± 0.407 ml/g (Fig. 3), statistically different (\( p < 0.001 \), one-way ANOVA followed by Duncan’s test) from that obtained from control CP.

**Elimination Clearance of Taurine and Formycin B from CSF.** To determine whether the induction of inflammation in the CSF affects elimination from the CSF in case of other carrier-mediated transports, CSF elimination in the presence and absence of experimental inflammation was examined using two model substrates, taurine, and formycin B. A previous study indicated that taurine was eliminated by a \( \text{Na}^+ \)-dependent \( \beta \)-amino acid transporter from the CSF via the CP (Chung et al., 1994, 1996). When 5 ng of taurine was injected intraventricularly, the elimination clearance for taurine from the CSF was found to be 50.5 ± 13.7 \( \mu \)l/min (Fig. 4A), similar to that reported in a previous study (69.5 ± 27.7 \( \mu \)l/min; Chung et al., 1996). The elimination clearance of taurine was reduced to 22.8 ± 2.72 \( \mu \)l/min (Fig. 4A; \( p < 0.05 \) by one-way ANOVA followed by Duncan’s test) in LPS administered rats. The reduction in taurine clearance was returned (Fig. 4A; 58.9 ± 7.48 \( \mu \)l/min) to the control level of clearance when LPS and N-nitro-l-arginine were coadministered. Similar to the case of BPC, when N-nitro-l-arginine was administered to control rats, the elimination clearance for taurine (Fig. 4A; 50.5 ± 6.24 \( \mu \)l/min) was similar to that obtained in control rats.

Formycin B, a nonmetabolizable analog of inosine, has been shown to be eliminated by the \( \text{Na}^+ \)-dependent nucleoside transporter (N1) from the CSF via the CP (Wu et al., 1992). When 6.7 ng of formycin B was injected intraventricularly, the elimination clearances for formycin B were 8.23 ± 1.26 \( \mu \)l/min and 11.4 ± 6.77 \( \mu \)l/min in control and LPS administered rats, respectively (Fig. 4A), indicating that the induction of experimental inflammation in the CSF had no effect on the elimination of formycin B from CSF. The clearance value for formycin B was similar to that reported in a previous study (Wu et al., 1993).

**Taurine and Formycin B Uptake into Isolated CP.** Taurine and formycin B uptake into ATP-depleted CP was also examined in the presence of an inwardly directed \( \text{Na}^+ \) gradient (Wu et al., 1993; Chung et al., 1994). The temporal profiles of taurine and formycin B uptake into the CP were found to be linear up to 20 and 5 min, respectively (data not shown). Therefore, 20 and 5 min incubation time were used for taurine and formycin B, respectively. The uptake of taurine into the CP obtained from control rats was 5.04 ± 0.923 ml/g, consistent with a previous report (i.e., 4.85 ± 0.68 ml/g; Chung et al., 1994). Taurine uptake into the CP obtained from LPS administered rats and control CP pretreated with SNP was 2.88 ± 0.699 and 3.22 ± 0.854 ml/g, respectively, was significantly less than the control value (one-way ANOVA with Duncan’s test, for both cases \( p < 0.001 \) from the uptake value of control CP; Fig. 4B). Formycin B uptake into CP obtained from control rats, CP from LPS administered rats, and control CP pretreated with SNP was 2.09 ± 0.563, 1.34 ± 0.461, 1.77 ± 0.948 ml/g, respectively (Fig. 4B). Unlike BPC and taurine, the uptake of formycin B into CP did not appear to be affected by these treatments.

**Discussion**

Increased levels of penicillins, including BPC, in the CSF have been reported in experimental inflammation in the CSF (Lithander, 1965; Spector and Lorenzo, 1974) as well as in human meningitis involving bacterial infections (Thrupp et al., 1965). Spector and Lorenzo (1974) have postulated that the increased levels may be linked to an increased penetration and/or the suppressed elimination of BPC in the CSF. The kinetics of BPC after an injection of the drug to the lateral ventricle was examined in this study, and an elevation in drug concentration in the CSF was observed. Since BPC is administered directly to the lateral ventricle, an increased penetration of the drug from the systemic circulation to the CSF is not likely to be the primary cause of elevated levels of BPC in the CSF. In addition, the uptake of BPC was reduced in CP samples obtained from inflammation-induced rats. Considering these facts, the elevated level of BPC may be primarily mediated by a reduced elimination from the CSF, rather than an increase in permeability to the CSF for BPC in the case of experimental inflammation.

In previous reports, the permeability of the blood brain barrier was found to be increased during experimental inflammation (Wispelwey B et al., 1988; Roord et al., 1994; Jaworowicz et al., 1998; Xia et al., 2001). As a result, it has been suggested that a similar increase in permeability may occur for the interface between blood and CSF.
during the inflammation in the CSF. In this study, however, inulin clearance was unaffected by the induction of inflammation in the CSF, suggesting that the diffusional permeability of the blood-CSF barrier was not increased by CSF inflammation. Typically, an increase in permeability is associated with the administration of a very high dose of LPS. For example, Boje (1995, 1996) reported an increase in the permeability of the blood brain barrier after an administration of 25 and 200 \( \mu \text{g} \) of LPS. In comparison, a relatively small dose of LPS to the cisterna magna was used (i.e., 50 ng of LPS per rat) in this study. Thus, the difference in the LPS dose may have contributed to the discrepancy in the alteration of the permeability of barriers between the blood and the central nervous system.

The primary focus of this study was the impact of NO overproduction on the functional activities of carrier-mediated transport systems for three model compounds in the CP epithelium and on the relevant pharmacokinetics in the CSF. Based on both in vitro and in vivo experimental systems, our data indicate that more than one transport system appear to be suppressed in the presence of endogenous NO overproduction or an excessive level of an NO donor. The injection of LPS was accompanied by a significant increase in WBC count and the level of nitrogen oxides (\( p < 0.05 \)) in the CSF, indicating that NO production was indeed induced as the result of the inflammation. In other experimental systems, the presence of nitrogen oxides has been related to alterations in the functional activity of transport systems such as those for reduced-folate and glutamate (Trotti et al., 1996; Smith et al., 1999). In these studies, however, transport function was typically studied using exogenously added NO. To our knowledge, this study represents the first example in the literature wherein inflammation is directly linked to the functional impairment of transporters as the result of the induction of NO synthase.

It should be noted that an NO-mediated effect may not always be associated with the depression in the functional activity of transporters, since the presence of NO has no effect on the uptake of formycin B into the CP or its elimination from the CSF. The underlying mechanisms that affect transport function in the presence of NO appear to be complex and, thus, further investigation is warranted for a complete understanding of this process.

Smith and coworkers (1999) demonstrated that the addition of an NO donor [i.e., \( \text{S-nitroso-}N\text{-acetylpenicillamine (SNAP)} \)] had no effect on the functional activity of taurine uptake in human retinal pigment epithelial cells. In contrast, the accumulation of taurine was depressed in the presence of SNP in ATP-depleted CP. The mechanism for the discrepancy in taurine transport was not directly studied. Differences in experimental design (viz., cell culture versus ATP-depleted CP) and/or types of NO donor used may, in part, explain this discrepancy. For example, NO donors, which rapidly release NO (e.g., SNP), do not effectively form peroxynitrite, a potent oxidant in biological systems (Trotti et al., 1996). Based on a headspace assay of NO (Chung and Fung, 1990; Chung et al., 1992), the rate of spontaneous NO generation was significantly faster for SNP (Kowaluk and Fung, 1990), the NO donor used by Smith and coworkers (1999), than for SNP (Kowaluk et al., 1992). Therefore, depending on the type of NO donors, the proportion of peroxynitrite and nitric oxide may vary depending on experimental designs and, thus, the extent of functional impairment of transporters in the presence of these NO donors.

SNP byproducts (e.g., cyanide, ferrocyanide, or ferricyanide ions) have been reported to affect protein function (Dulak et al., 2000). In this study, we did not directly study the potential involvement of SNP byproducts in the functional impairment of model transport systems. However, the LPS administration study suggests that the endogenous production of NO is involved in the functional impairment of BPC and taurine transport. Since the treatment is generally considered to be absent for typical SNP byproducts (e.g., cyanide, ferrocyanide, or ferricyanide ions) other than NO, the involvement of SNP byproducts in functional impairment is not likely to be the primary mechanism.

Despite the fact that significantly higher levels of nitrogen oxides are present in inflammation-induced CSF, the specie(s) of nitrogen oxides responsible for the functional impairment of carriers of BPC...
and taurine is (are) not clear. Since both NO and peroxynitrite may affect certain protein functions, any of these nitrogen oxides may be involved in the reduction of functional activities of transporters. In a preliminary experiment, the functional activity of the Na⁺-dependent taurine transporter in the presence of SNP was studied using rat renal brush border membrane vesicles. Interestingly, we have found that the addition of superoxide dismutase reversed the effect of SNP. Since the superoxide anion is required for the formation of peroxynitrite from NO, this preliminary observation indicates that nitration rather than other mechanisms (e.g., direct chemical interaction of the transporter with NO, cyanide ion, ferricyanide, or ferricyanide ions) may be responsible for the functional impairment of the taurine transporter in the presence of SNP.

In summary, the elimination clearance of BPC and taurine from the CSF was reduced as the result of the induction of experimental inflammation in the CSF but could be returned to control levels by pretreatment with N-nitro-l-arginine in inflammation-induced rats. The uptake of BPC and taurine was suppressed in CP samples obtained from inflammation-induced rats and control CP pretreated with SNP, an NO donor, indicating that more than one transport system is functionally reduced in the presence of pathological levels of NO. Previously, the concentration of BPC in the CSF was elevated in experimental and human meningitis. Therefore, the sensitivity of NO to the BPC transport system in the blood-CSF barrier may be responsible, at least in part, for the altered pharmacokinetics in CSF during meningitis.

**References**


