ABSTRACT

Cumulative evidence suggests that several organic anions are actively effluxed from the brain to the blood across the blood-brain barrier (BBB). We examined the possibility of the presence of primary active transporters for organic anions (multidrug resistance associated protein (MRP) and canalicular multispecific organic anion transporter (cMOAT)) on the BBB by measuring the ATP-dependent uptake of 2,4-dinitrophenyl-S-glutathione (DNP-SG) and leukotriene C₄ (LTC₄) into membrane vesicles prepared from a cell line derived from mouse brain capillary endothelial cells (MBEC4). The ATP-dependent uptake of DNP-SG into the membrane vesicles was osmotically sensitive and was also supported by GTP, but not by AMP or ADP. An ATPase inhibitor, vanadate, blocked the ATP-dependent uptake of DNP-SG. The ATP-dependent uptake process was saturable, with \( K_m \) values of 0.56 and 0.22 \( \mu \text{M} \), and \( V_{\text{max}} \) values of 5.5 and 27.5 pmol/min/mg protein for DNP-SG and LTC₄ respectively. Northern and Western blot analyses showed the expression of murine MRP but not cMOAT in MBEC4 cells. Western blot analysis of the rat cerebral endothelial cells indicated the expression of protein(s) that is detectable with MRP1, an antibody against MRP. These results, together with previous findings that both DNP-SG and LTC₄ are good ligands for MRP, suggest that MRP is responsible for the unidirectional, energy-dependent efflux of organic anions from the brain into the circulating blood across the BBB.

The brain uptake of compounds is restricted by the BBB that acts as a shield to protect the brain. The BBB is formed by the tight junction, an anatomical feature of brain capillary endothelial cells, which connects them to each other (Bradbury, 1979; Pardridge, 1991; Rapoport, 1976). Because the molecules in the circulating blood have to be transported across the cerebral endothelial cells transcellularly to enter the brain due to the presence of the tight junction and the paucity of fenestra or pinocytotic vesicles, the brain penetration of compounds particularly those with high hydrophilicity and/or high molecular weight is restricted (Bradbury, 1979; Pardridge, 1991; Rapoport, 1976). Although several cationic or neutral compounds are lipophilic, their penetration into the brain is restricted by P-gp, a primary active transporter on the luminal membrane of the cerebral endothelial cells that extrudes cationic or neutral compounds from the brain into the circulating blood (Tamai and Tsuji, 1996; Tatsuta et al., 1992; Schinkel et al., 1994).

In addition, cumulative evidence suggests that several organic anions are transported from the brain to the blood across the BBB (Suzuki et al., 1997; Leininger et al., 1991) indicated that the elimination of 1-naphthyl-\( \beta \)-D-glucuronide after microinjection into the cerebral cortex is mediated by a saturable process. We also analyzed the time profiles of the brain concentrations of an anionic \( \beta \)-lactam antibiotic, cefodizime after i.v. administration and found that this compound is actively transported from the brain to the blood across the BBB (Matsushita et al., 1991). The fact that both 1-naphthyl-\( \beta \)-D-glucuronide and cefodizime are substrates for the primary active transporter for organic anions located on the bile canalicular membrane (cMOAT) (Kobayashi et al., 1990; Kusuhara et al., in press; Sathirakul et al., 1994; Yamazaki et al., 1993) prompts us to hypothesize that cMOAT and/or its related transporter(s) may be expressed on the BBB.

Our purpose is to examine this hypothesis in an \textit{in vitro} model for studying ligand transport across the BBB. As a cell line, we used MBEC4 cells, established by infecting isolated mouse cerebral endothelial cells with SV40 (Tatsuta et al., 1992). Because 1) MBEC4 cells retain properties specific to cerebral endothelial cells in the brain such as the expression of acetyl low-density lipoprotein receptors, \( \gamma \)-glutamyl

ABBREVIATIONS: BBB, blood-brain barrier; CNS, central nervous system; MRP, multidrug resistance associated protein; P-gp, P-glycoprotein; DNP-SG, 2,4-dinitrophenyl-S-glutathione; LTC₄, leukotriene C₄; hMRP, human multidrug resistance associated protein; cMOAT, canalicular multispecific organic anion transporter; ATP, adenosine 5’-triphosphate; AMP, adenosine 5’-monophosphate; GTP, guanosine 5’-triphosphate; SDS, sodium dodecyl sulfate.
transpeptidase and alkaline phosphatase, 2) a monolayer of MBE4C cells with little paracellular leakage can be formed with the localized luminal expression of P-gp, this cell line has been used to characterize the transport properties of P-gp located on the BBB (Tatsuta et al., 1992). In our study, we examined the ATP-dependent uptake of DNP-SG and LTC<sub>4</sub>, typical substrates for primary active transporters (such as cMOAT and MRP) (Lautier et al., 1996; Lue et al., 1996a; Keppler and Arias, 1997; Kusuhara et al., 1993) into membrane vesicles prepared from MBE4C cells. In addition, we examined the expression of MRP along with that of cMOAT by Northern and Western blot analyses.

Materials and Methods

Chemicals. Unlabeled and <sup>3</sup>H]DNP-SG (50.0 μCi/nmol) were synthesized enzymatically using [glycine-2-<sup>14</sup>]<sup>1</sup>H]glutathione (New England Nuclear, Boston, MA), 1-chloro-2,4-dinitrobenezene and glutathione S-transferase (Sigma Chemical Co., St. Louis, MO) as described previously (Kobayasi et al., 1990), and the purity was checked by thin-layer chromatography. [14,15,19,20-<sup>3</sup>H]LTC<sub>4</sub> (128 μCi/nmol), [125]I]sheep anti-mouse immunoglobulin and [125]I]sheep anti-rat immunoglobulin antibodies were purchased from Amersham International (Buckinghamshire, UK). Unlabeled LTC<sub>4</sub> was purchased from Sigma, Rat anti-hMRP antibody, MrPr1 and mouse anti-human P-gp antibody, C219 were purchased from Kamiya Biomedicals (Tukwila, WA) and Centocor (Malvern, PA), respectively. All other chemicals were commercially available, of reagent grade and used without further purification.

Cell lines. MBE4C cells, established by immortalizing the isolated mouse brain capillary endothelial cells by SV40 infection (Tatsuta et al., 1992), were used in our study. The cells were maintained in Dulbecco’s modified Eagle’s medium (low glucose) supplemented with 10% fetal bovine serum in 5% CO<sub>2</sub>-95% air at 37°C. Media and reagents were purchased from Eurobio (Saint Martin, France), Immunoprecipitation grade stock solutions of sodium and potassium chloride, 0.5 mCi/ml [35S]methionine, 0.5 mCi/ml [35S]cysteine, and 0.5 mCi/ml [35S]lysine (ICN Biochemicals, Sunnyvale, CA) were used without further purification.

Membrane vesicle preparation. All steps were performed at 0 to 4°C. Membrane vesicles were prepared by nitrogen cavitation from MBE4C cells according to the method described previously (Fujii et al., 1994) with minor modification. Cell monolayers were washed and scraped into phosphate-buffered saline. The cells were washed by centrifugation (4000 × g for 10 min) in phosphate-buffered saline and then in buffer A (10 mM Tris-HCl, 250 mM sucrose, and 2 mM CaCl<sub>2</sub>, pH 7.5). The pellet was stored at -100°C until required. The defrosted cells were equilibrated at 4°C under a nitrogen pressure of 63 kg/cm<sup>2</sup> for 30 min, and then depressurized rapidly. EDTA (final concentration 1 mM) and 3 volumes of buffer B (10 mM Tris-HCl and 250 mM sucrose, pH 7.5) were added to the lysed cell suspension, then centrifuged at 1000 × g for 10 min at 4°C to remove nuclei and unlysed cells. The supernatant was layered on a 25% sucrose cushion (10 mM Tris-HCl, 35% sucrose and 1 mM MgSO<sub>4</sub>) and centrifuged for 45 min at 4°C. The resulting membrane vesicle fraction was collected by centrifugation (100,000 × g for 45 min at 4°C). The vesicle pellet was resuspended in buffer B using a 25-gauge needle. Vesicles were stored at -100°C until required. The orientation of membrane vesicles was determined by examining the nucleotide pyrophosphatase in the presence and absence of 1% Triton X with p-nitrophenylthiophosphate 5'-monophosphate as the substrate (Bohme et al., 1994).

The transport study was performed using the rapid filtration technique described in a previous report (Ishikawa et al., 1990). Transport medium (10 mM Tris-HCl, 250 mM sucrose and 10 mM MgCl<sub>2</sub>, pH 7.4), containing radiolabeled compounds (15 μl), with or without unlabeled substrate, was preincubated at 37°C for 3 min and then rapidly mixed with 5 μl membrane vesicle suspension (10 μg protein) with or without 5 mM ATP and ATP-regenerating system (10 mM creatine phosphate and 100 μg/ml creatine phosphokinase).

In some instances, ATP was performed by AMP, ADP or GTP. The effect of vanadate (100 μM) on ATP-dependent transport without ATP-regenerating system was also examined. The transport reaction was stopped by the addition of 1 ml ice-cold buffer containing 250 mM sucrose, 100 mM NaCl and 10 mM Tris-HCl (pH 7.4). The stopped reaction mixture was filtered through a 0.45 μm GVWP filter (Millipore, Bedford, MA) and washed twice with 5 ml stop solution. Radioactivity retained on the filter was determined using a liquid scintillation counter (LSC-5500, Aloka, Tokyo, Japan). All the uptake studies were performed in triplicate or quadruplicate using one preparation. Analysis of variance followed by Fisher’s t test was used to determine the significance of differences between the means of two groups, with P < 0.05 as the minimum level of significance.

The kinetic parameters for the uptake of DNP-SG and LTC<sub>4</sub> into membrane vesicles prepared from MBE4C cells were estimated from the following equation:

\[ V_0 = V_{max} \times \frac{S}{(K_m + S)} + P_{dir} \times S \]

where \( V_0 \) is the initial uptake rate of the substrate (pmol/min/mg protein), \( S \) is the substrate concentration in the medium (μM), \( K_m \) is the Michaelis-Menten constant (μM), \( V_{max} \) is the maximum uptake rate (pmol/min/mg protein) and \( P_{dir} \) is the clearance for the nonspecific uptake (μmol/min/mg protein). The equation was fitted to the ATP-dependent transport velocity, which was obtained by subtracting the transport velocity in the absence of ATP from that in its presence, by an iterative nonlinear least-squares method using a MULTI program (Yamaoka et al., 1981) to obtain estimates of the kinetic parameters. The input data were weighted as the reciprocal of the observed values, and the Damping Gauss Newton algorithm method was used for fitting. The fitted line was converted to the \( V_0/S \) vs \( V_0 \) form (Eadie-Hofstee plot).

Isolation of brain capillaries. Capillaries were isolated from Wistar rats (Male, 240-270 g; Nihon Ibagakyo, Tokyo, Japan) using the method described previously (Pardridge et al., 1985) with minor modification. After decapitation, the cerebral was removed quickly, rinsed with ice-cold buffer containing 122 mM NaCl, 25 mM NaHCO<sub>3</sub>, 0.1 mM d-glucose, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub> and 10 mM HEPES (pH 7.4). The miniced cerebrum was homogenized with 2 volumes of buffer using a glass/ Teflon Potter homogenizer for five periods of 1 min each at 200 rpm. The homogenate was filtered through a 32-μm nylon mesh. Trapped brain capillaries were stored at -80°C until assayed.

Northern blot analysis. Northern hybridization was performed as described previously (Ito et al., 1996-1998). The cDNA fragment encoding the carboxy-terminal ABC region of murine MRP was amplified from MBE4C cell RNA by RT-PCR using degenerated PCR primers as described previously (Ito et al., 1998). The cDNA fragments containing the linker region of murine mdr1a and mdr1b (nucleotide 1343-1476 and 1921-2088, respectively; Creop et al., 1989) were also prepared by RT-PCR, using RNA from ddy mouse liver as a template. The amplified PCR products were subcloned into the EcoR V site of pBluescript II SK(-) and then the sequence was determined. The cDNA fragments were excised by digestion with EcoRI and HindIII as the probe.

Total RNA was prepared from MBE4C cells by a single-step guanidinium thiocyanate procedure (Ito et al., 1996-1998). Poly(A)<sup>+</sup> RNA was purified using oligotex dT<sub>30</sub> (Takara Shuzo). Five micrograms of poly(A)<sup>+</sup> RNA were separated on 0.7% agarose gel containing 3.7% formaldehyde and transferred to a nylon membrane (Bio-dyne, Pall, Glen Cove, NY), before fixation by baking for 2 hr at 80°C. Blots were prehybridized in hybridization buffer containing 4 x SSC, 5 x Denhardt’s solution, 0.2% SDS, 0.1 mg/ml sonicated salmon sperm DNA and 50% formamide at 42°C for 4 hr. Hybridization was performed overnight in the same buffer containing 10<sup>6</sup> cpm/ml [32P]labeled cDNA probes prepared by a random primed labeling method (Rediprime, Amersham International). The hybridized membrane was washed in 2 x SSC and 0.1% SDS at room temperature for

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20 min, followed by washing in 2x SSC and 0.1% SDS at 55°C for 20 min and then in 0.1x SSC and 0.1% SDS at 55°C for 20 min. Filters were exposed to Fuji imaging plates (Fuji Photo Film, Kanagawa, Japan) for 3 hr at room temperature and analyzed by a BAS imaging analyzer (Fuji Photo Film). In addition, the expression of cMOAT was examined by RT-PCR according to the method described previously (Ito et al., 1998).

Western blot analysis. A total of 25 µg of the proteins of MBEC4 membrane vesicles and rat brain capillary was fractionated on a 7.5% (w/v) polyacrylamide slab gel containing 0.1% (w/v) SDS and then transferred onto a nitrocellulose filter by electroblotting. The filter was incubated for at least 1 hr in 10 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl, 0.05% Tween-20 and 5% (w/v) bovine serum albumin for MRPr1 and in 10 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl, 0.05% Tween-20 and 5% (w/v) milk powder for C219 to prevent nonspecific binding of antibodies. Then, it was incubated with MAb (MRPr1, 1:50; C219, 1:100) for 12 hr, and with [125I] sheep anti-rat immunoglobulin and [125I] sheep anti-mouse immunoglobulin antibodies for 1 h at room temperature in the same buffer. Filters were exposed to Fuji imaging plates for 3 h at room temperature and analyzed by a BAS imaging analyzer.

Results

Uptake of [3H]DNP-SG and [3H]LTC4 into membrane vesicles. Determination of nucleotide pyrophosphatase in the presence and absence of 1% Triton X revealed that 65% of the membrane vesicles were inside out. Figure 1 shows the time-profiles for the uptake of [3H]DNP-SG into membrane vesicles. Osmotic sensitivity was studied by examining the uptake of [3H]DNP-SG into membrane vesicles prepared from MBEC4 cells in the presence or absence of 5 mM ATP. The uptake of both ligands into membrane vesicles was stimulated by ATP (fig. 1).

Osmotic sensitivity of the uptake of [3H]DNP-SG into membrane vesicles. Osmotic sensitivity was studied by examining the uptake of [3H]DNP-SG into membrane vesicles in the presence of several concentrations of sucrose in the medium to confirm that a major part of the accumulation can be accounted for by transport into the intravesicular space, but not by binding to the vesicle surface. As shown in figure 2, the uptake of [3H]DNP-SG at steady-state was reduced as the sucrose concentration in the medium increased. The y-intercept for the relationship between the amount of DNP-SG associated with the vesicles versus the reciprocal of the sucrose concentration in the medium was 5.5 µl/mg protein (fig. 2). The amount of DNP-SG bound to the vesicle surface was less than 10% of the total vesicle uptake, if the transport experiment was performed in isotonic medium.

Nucleotide specificity of [3H]DNP-SG uptake into membrane vesicles. Nucleotide specificity of [3H]DNP-SG uptake was examined by replacing ATP by other nucleotides. As shown in table 1, DNP-SG uptake was most efficient with ATP, but not ADP or AMP. GTP was to some extent also able to stimulate the uptake of [3H]DNP-SG into membrane vesicles prepared from MBEC4 cells. One hundred micromolar vanadate, an inhibitor of ATPases, reduced the ATP-dependent uptake of [3H]DNP-SG to 60% of the control value.

Kinetics of the uptake of [3H]DNP-SG and [3H]LTC4 into membrane vesicles. To obtain the kinetic parameters...
TABLE 1
Nucleotide specificity for the uptake of [3H]DNP-SG into membrane vesicles from MBEC4 cells

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>Uptake (μmol/mg protein)</th>
<th>%Relative Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (+regenerating system)</td>
<td>19.3 ± 0.4</td>
<td>100*</td>
</tr>
<tr>
<td>ATP (−regenerating system)</td>
<td>17.7 ± 0.3</td>
<td>100*</td>
</tr>
<tr>
<td>ATP+vanadate</td>
<td>10.4 ± 0.5</td>
<td>58*</td>
</tr>
<tr>
<td>ADP</td>
<td>2.5 ± 0.1</td>
<td>14*</td>
</tr>
<tr>
<td>AMP</td>
<td>2.5 ± 0.2</td>
<td>14*</td>
</tr>
<tr>
<td>GTP</td>
<td>14.2 ± 0.6</td>
<td>80*</td>
</tr>
<tr>
<td>None</td>
<td>2.3 ± 0.2</td>
<td>13*</td>
</tr>
</tbody>
</table>

After a 3-min preincubation with nucleotide (5 mM), membrane vesicles were incubated with [3H]DNP-SG (1 μM) for 5 min at 37°C. Uptake was determined as described in “Materials and Methods.” ATP (+regenerating system) shows uptake in the presence of ATP and its regenerating system (5 mM ATP, 10 mM creatine phosphate and 100 μg/ml creatine phosphokinase). The data represent mean ± S.E. (n = 4) from one preparation performed in quadruplicate. Analysis of variance followed by Fisher’s t test was used to determine the significance of differences among groups.

* P < .05 (relative to ATP (+regenerating system)).

Discusson

We examined the presence of an efflux transporter on the BBB for organic anions by examining the transport of DNP-SG and LTC4, typical substrates of cMOAT and MRP, into membrane vesicles prepared from MBEC4 cells. ATP stimulated the uptake of [3H]DNP-SG and [3H]LTC4 into membrane vesicles (fig. 1). This ATP-dependent uptake was osmotically sensitive (fig. 2), suggesting that the major part of the uptake of [3H]DNP-SG is actually due to uptake, and not adsorption to membrane vesicles. Because 1) ATP-dependent uptake of [3H]DNP-SG was inhibited by vanadate and 2) ADP or AMP did not stimulate the uptake of [3H]DNP-SG (table 1), the uptake of [3H]DNP-SG requires hydrolysis of ATP. Not only ATP but also GTP could stimulate the uptake of [3H]DNP-SG into membrane vesicles (table 1), which is consistent with a previous observation in rat bile canalicular membrane vesicles (Ballatori and Truong, 1995; Kobayashi et al., 1996). Nonlinear regression analysis yielded K_m values of 0.557 ± 0.066 μM and 0.221 ± 0.047 μM, and V_max values of 5.47 ± 2.66 pmol/min/mg protein and 27.5 ± 3.9 pmol/min/mg protein for DNP-SG and LTC4, respectively. The fit in figure 3b seems poor. Due to the saturation, the transport in the presence and absence of ATP was similar at higher concentrations of LTC4 and this fact resulted in the poor fit along with the large deviations in V_max.

Northern and Western blot analyses. The expression of cMOAT and MRP in MBEC4 cells was examined by Northern blot analysis. As shown in figure 4, murine MRP probe hybridized with poly A + RNA from MBEC4 cells at the same location (6.0 kb) as that reported previously (Stride et al., 1996). In contrast, no band was observed if a cMOAT probe was used, although two bands (6.0 and 8.2 kb) were detected in BALB/C mouse liver (data not shown). Furthermore, RT-PCR failed to amplify the cMOAT cDNA fragment with MBEC4 cDNA.

Figure 4 also shows that MRPr1 reacts with an approximately 200-kDa protein on membranes isolated from MBEC4 cells. The length of the protein reacting with MRPr1 was comparable to that reported previously (Wijnholds et al., 1997) demonstrated that 190-kDa protein bands detected by MRPr1 in tissues such as heart, testis, stomach, erythrocytes and bone marrow-derived mast cells of wild type mice were absent in MR knock-out mice. Although MRPr1 reacted with a protein expressed on rat cerebral endothelial cells, the length of the band is shorter than that of MRP, which might be accounted for by the considering species difference.

Northern blot analysis revealed that mrd1b, but not mdr1a, was detectable in MBEC4 cells (data not shown), which is consistent with a previous observation (Tatsuta et al., 1992). In addition, C219 cross-reacted with a protein (150-170 kDa) on membrane vesicles from MBEC4 cells.
Kinetic parameters for the uptake of DNP-SG and LTC₄ should be compared in several cell lines. Because 1) the ATP-dependent uptake of these glutathione conjugates into membrane vesicles from MBEC4 cells consisted of one saturable component (fig. 3), and 2) Northern and Western blot analyses indicated the expression of MRP but not cMOAT in hMRP-transfected HeLa cells (Loe et al., 1996b), these results indicate that MRP/cMOAT activity is associated with the plasma membrane of MBEC4 cells. Because Northern and Western blot analyses suggest the expression of MRP in MBEC4 cells, it is plausible that the transport activity is mediated, at least in part, by MRP. In principle, the ATP-dependent uptake of ligands can be ascribed to uptake into inside out membrane vesicles that account for 65% of the prepared vesicles, and therefore, the transport observed in vitro represents efflux of ligands from cells under physiological conditions.

Collectively, the transport properties of primary active transporter(s) expressed on L1210 and MBEC4 cells resemble each other kinetically. Kinetic parameters determined in these mouse cell lines, however, were different from those reported for hMRP. Jedlitschky et al. (1996) determined the kinetic parameters for the uptake of DNP-SG and LTC₄ in hMRP-transfected HeLa cells and found that the affinity of LTC₄ for hMRP is approximately 40-fold higher than that of DNP-SG (3.6 μM). Because the Vₘₐₓ values of LTC₄ and DNP-SG for hMRP are 100 and 409 pmol/min/mg protein in hMRP-transfected HeLa cells, respectively, the clearance for the uptake at tracer concentrations (CL_uptake) defined as the Vₘₐₓ/Kₘₑₙ for LTC₄ (1031 μl/min/mg protein) is much higher than that of DNP-SG (114 μl/min/mg protein) (Jedlitschky et al., 1996). Although the CL_uptake of LTC₄ (124 μl/min/mg protein) was approximately 13-fold higher than that of DNP-SG (9.82 μl/min/mg protein) in MBEC4 vesicles, this difference is ascribed predominantly to the difference in Vₘₐₓ values between the two ligands (27.5 pmol/min/mg protein and 5.47 pmol/min/mg protein for LTC₄ and DNP-SG, respectively). Collectively, these results suggest that the transport characteristics of MRP may be different in mice and humans. Such a difference in the transport properties of MRP between mice and humans has been reported previously. Stride et al. (1997) examined the resistance of murine and human MRP-transfected HEK293 cells to anti-tumor drugs and found that murine and human MRP conferred similar resistance profiles with the exception that only hMRP conferred resistance to anthracyclines. In addition, the accumulation of [³²P]vincristine and [³²P]VP-16 was reduced and efflux of [³²P]vincristine was increased in both murine and human MRP-transfectants, although only hMRP-transfectants displayed reduced accumulation and increased the efflux of [³²P]daunomycin (Stride et al., 1997).

Although expression of MRP in the brain was reported using RNase protection assay/Northern blot analysis (Flens et al., 1996; Stride et al., 1996), localization in the brain has not been examined. Western blot analysis revealed that MRP or closely related protein is expressed on the rat brain capillary (fig. 4). Although the localization of this protein on brain capillary endothelial cells (luminal or antiluminal) has not been clarified yet, the results of the present study are consistent with the hypothesis that MRP and/or its related protein is expressed on the rat brain capillary endothelial cells. Because 1) glucuronide and glutathione conjugates are substrates for MRP (Lautier et al., 1996; Keppler et al., 1996) and 2) UDP-glucuronosyltransferase and glutathione-S-transferase are expressed in brain parenchyma and cerebral endothelial cells (Ghersi-Egea et al., 1994), it is plausible that the conjugated metabolites formed in the CNS are transported to the blood across the BBB. The presence of such a sequential detoxification of xenobiotics by metabolic enzymes and efflux transporter(s) in the liver has been suggested (Ishikawa, 1992). Based on the results of the transport studies in normal rats (such as Sprague-Dawley and Wistar strains) and mutant rats whose cMOAT function is hereditarily defective (such as Eisai hyperbilirubinemic and TR strains), we and others have demonstrated that the substrate for cMOAT includes glutathione conjugates (such as DNP-SG, LTC₄ and glutathione disulfide) and glucuronide conjugates (such as glycyrrhizin, glucuronides of bilirubin and 6-hydroxy 5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole dihydrochloride and liquiritigenin) (Lautier et al., 1996; Leier et al., 1994; Loe et al., 1996a; Keppler and Arias, 1997; Kusuhara et al., in press; Oude-Elferink et al., 1995; Yamazaki et al., 1993). In addition, we demonstrated that the nonconjugated compounds such as pravastatin, temocaprilat, cefozamide and the carbonate form of CPT-11 and SN-38 are extruded via cMOAT (Kusuhara et al., in press; Yamazaki et al., 1993). Because the substrate specificity of cMOAT and MRP is similar (Lautier et al., 1996; Loe et al., 1996a; Keppler et al., 1997; Kusuhara et al., in press; Oude-Elferink et al., 1995; Yamazaki et al., 1993), it is plausible that the penetration of these compounds into the brain is restricted by MRP expressed on cerebral capillary endothelial cells. Although Cornford et al. (1985) and Masereeuw
et al. (1994) along with Takasawa et al. (1997) reported active efflux of valproic acid and 3'-azide-3'-deoxythymidine across the BBB by examining the time-dependent change in the brain uptake index and by examining the after administration into the cerebral cortex, respectively, it remains to be established whether these ligands are actively transported across the BBB via MRP.

In conclusion, our study demonstrated the expression of MRP on MBEC4 cells, retaining BBB properties. It is possible that the expression of such efflux transporter(s) along with the presence of metabolic enzymes endows cerebral endothelial cells with the ability to detoxify xenobiotics thereby providing a blood-brain barrier function.

References


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