ATP-dependent transport of organic anions into isolated basolateral membrane vesicles from rat intestine

Takahiro Shoji, Hiroshi Suzuki, Hiroyuki Kusuhara, Yuka Watanabe, Shingo Sakamoto, and Yuichi Sugiyama. ATP-dependent transport of organic anions into isolated basolateral membrane vesicles from rat intestine. Am J Physiol Gastrointest Liver Physiol 287: G749–G756, 2004. First published June 24, 2004; 10.1152/ajpgi.00065.2003.—The mechanism for the cellular extrusion of organic anions across the intestinal basolateral membrane was examined using isolated membrane vesicles from rat jejunum, ileum, and colon. It was found that 17β-estradiol 17β-D-glucuronide (E2 17βG) is taken up in an ATP-dependent manner into the basolateral membrane vesicles (BLMVs) but not into the brush-border or microsomal counterparts. The ATP-dependent uptake of E2 17βG into BLMVs from jejunum and ileum was described by a single component with a $K_m$ value of 23.5 and 8.31 μM, respectively, whereas that into the BLMVs from colon was described by assuming the presence of high ($K_m$ = 0.82 μM)- and low-affinity ($K_m$ = 35.4 μM) components. Taurocholate, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridyl)-methyl) benzothiazole glucuronide and taurohicolosulate sulfate, but not leukotriene C4, were significantly taken up by the BLMVs from colon was described by assuming the presence of high ($K_m$ = 0.82 μM)- and low-affinity ($K_m$ = 35.4 μM) components. Taurocholate, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridyl)-methyl) benzothiazole glucuronide and taurohicolosulate sulfate, but not leukotriene C4, were significantly taken up by the BLMVs. In addition to such substrate specificity, the inhibitor sensitivity of the ATP-dependent transport in BLMVs was similar to that of rat multidrug resistance-associated protein 3 (Mrp3), which is located on the basolateral membrane of enterocytes. Together with the fact that the rank order of the extent of the expression of Mrp3 (jejenum < ileum < colon) is in parallel with that of the extent of the transport of ligands, these results suggest that the ATP-dependent uptake of organic anions into isolated intestinal BLMVs is at least partly mediated by Mrp3.

intestinal transport; basolateral membrane; organic anion transport; bile acid transport; multidrug resistance-associated protein

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THE SMALL INTESTINE IS THE SITE OF ABSORPTION OF MANY ENDOGENOUS AND EXOGENOUS COMPOUNDS. ON THE BASIS OF THE RESULTS OBTAINED FROM IN VIVO STUDIES, SMALL INTESTINAL PERFORATION STUDIES, IN VITRO STUDIES USING USSING CHAMBERS, AND/OR EVERTED SACS, IT HAS BEEN SUGGESTED THAT TRANSPORTERS ARE INVOLVED IN THE ABSORPTION OF CERTAIN KINDS OF SUBSTANCES. MORE RECENTLY, TRANSPORTER MOLECULES LOCATED ON THE BRUSH-BORDER MEMBRANE OF THE INTESTINE HAVE BEEN CLONED, AND THEIR TRANSPORT PROPERTIES HAVE BEEN CHARACTERIZED. CONCERNING THE TRANSPORT OF ORGANIC ANIONS, IT HAS BEEN DEMONSTRATED THAT THE ILEUM APICAL Na+-DEPENDENT BILE ACID TRANSPORTER (ASBT) PREDOMINATES IN THE UPTAKE OF BILE SALTS INTO ENTEROCYTES (24). AS FAR AS NONBILE ACID ORGANIC ANIONS ARE CONCERNED, THE ROLE OF SEVERAL KINDS OF TRANSPORTERS HAS BEEN SUGGESTED. AMONG THEM, PEPT1, WHICH EXPRESSES MULTISUBSTRATE TRANSPORTER PROPERTIES IS RESPONSIBLE FOR THE ABSORPTION OF CERTAIN KINDS OF CEPHALOSPORIN ANTIBIOTICS, ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS, AND PRODRUGS OF NUCLEOSIDE ANALOGS (SUCH AS VALACYCLOVIR) (8, 10, 13, 18). HOWEVER, MONOCARBOXYLATE TRANSPORTER (MCT1) AND RAT ORGANIC ANION-TRANSPORTING POLYPEPTIDE 3 (OATP3) TRANSPORT ORGANIC ANIONS. ALTHOUGH MCT1 IS RESPONSIBLE FOR THE UPTAKE OF SMALL MOLECULES SUCH AS LACTATE (41), OATP3 HAS MUCH BROADER SUBSTRATE SPECIFICITY, AND ITS SUBSTRATES INCLUDE THYROID HORMONES, BILE ACIDS, 17β-ESTRADIOL 17β-D-GLUCURONIDE (E2 17βG), ESTROGEN-3-SULFATE, DEHYDROEPIANDROSTERONE SULFATE, BROMOSULFOPHThALEIN, AND BQ-123 (AN ANIONIC CYCLIC PEPTIDE WITH AN ENDOTHELIN RECEPTOR ANTAGONIST ACTIVITY) (1, 7, 42).


IN CONTRAST TO THE CUMULATIVE INFORMATION ON TRANSPORT ACROSS THE BRUSH-BORDER MEMBRANE, LITTLE IS KNOWN ABOUT TRANSPORT ACROSS THE BASOLATERAL MEMBRANE. IF WE CONSIDER THE FACT THAT THE CELLULAR ENTRY OF HYDROPHILIC ORGANIC ANIONS REQUIRES CERTAIN KINDS OF TRANSPORTERS, IT WOULD BE REASONABLE TO ASSUME THE PRESENCE OF SOME EFFLUX TRANSPORTERS ON THE BASOLATERAL MEMBRANE FOR EXTRUSION INTO THE BLOOD CIRCULATION. ON THE BASIS OF

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the previous finding that Mrp3/Abcc3, whose substrates include Mrp2 substrates and monovalent bile acids (2, 15, 16), is also expressed on the basolateral membrane of enterocytes (32), we assumed that such an ABC transporter may be involved in the absorption of a variety of compounds. In the present study, we have characterized the transport of organic anions across the intestinal basolateral membrane by examining whether ATP-dependent transport is observed in isolated basolateral membrane vesicles (BLMVs) from rat small intestine.

MATERIALS AND METHODS

Materials. [3H]Leukotriene C4 (LTC4; 130 μCi/ml), [3H]Estradiol 17β-D-glucuronide (E2; 44 μCi/ml), and [3H]taurocholate (3.0 μCi/ml) were purchased from PerkinElmer Life Science Products (Boston, MA). [3H]Taurolithocholate-3-sulfate (TLC-S) was synthesized from lithocholate-3-sulfate using 2-[3H]taurine (30.0 μCi/ml) as described previously (45). [14C]6-Hydroxy-5,7-dimethyl-2-methyl-a-amino-4-(3-pyridylmethyl) benzothiazole (E3040) glucuronide (84.5 μCi/ml) and unlabeled E3040 glucuronide and sulfate were prepared from E3040 (supplied by Eisai, Tsukuba, Japan) as described previously (40). Unlabeled DNP-SG was synthesized enzymatically using glutathione (Sigma, St. Louis, MO) and 1-chloro-2,4-dinitrobenzene (Sigma) and glutathione S-transferase (Sigma) as described previously (21). 4-Methylumbelliferone (4MU) glucuronide, 4MU sulfate, LTC4, α-naphthyl-β-D-glucuronide, TLC-S, and acivicin were from Sigma. Unlabeled taurocholate was purchased from Calbiochem (San Diego, CA).

Preparation of membrane vesicles. Preparation of rat intestinal epithelial cells was according to the method described by Lin et al. (23). Briefly, the segments were flushed and clamped with their distal end. They were filled with warm, oxygenated solution consisting of oxyxgenated, fortified PBS, 1.5 mM EDTA, 1 mM dithiothreitol, and 0.1% (wt/vol) BSA. The filled segments were incubated in oxygenated PBS at 37°C for 7.5 min in a shaker bath (100 oscillations/min). By gently squeezing the segments between fingers, cells were collected in a plastic beaker containing ice-cold solution consisting of (in mM) 122 NaCl, 25 NaHCO3, 4.72 KCl, 2.56 MgCl2, 1.2 KH2PO4, 2.5 CaCl2, and 1 dithiothreitol, with 1% BSA and gassed with 95% O2-5% CO2 to pH 7.4.

Membrane vesicles from the rat intestine were prepared according to the method described by Lin et al. (22). The mucosa was suspended in 15 vol of hypotonic buffer consisting of 1 mM Tris, 0.1 mM EDTA, 11.1 mM ATP, 5 mM AMP (●). Each point and vertical bar represents the mean ± SE of 3 determinations. The results (in pmol/mg protein) are given by dividing the amount of [3H]E217βG associated with BLMVs (pmol/mg protein) by the isotope concentration in the medium (pmol/μl).

Table 1. Activity of marker enzymes

<table>
<thead>
<tr>
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<th>Activity</th>
<th>Enrichment</th>
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<th>Enrichment</th>
<th>Activity</th>
<th>Enrichment</th>
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<tr>
<td>Na+-K+-ATPase</td>
<td>8.3±1.6</td>
<td>1.0±0.3</td>
<td>5.5±0.1</td>
<td>1.0±0.0</td>
<td>15.6±0.6</td>
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<td>BLMVs</td>
<td>34.1±1.3</td>
<td>4.1±0.8</td>
<td>7.4±0.1</td>
<td>1.3±0.0</td>
<td>22.0±0.4</td>
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<td>BBMVs</td>
<td>6.0±1.6</td>
<td>0.7±0.2</td>
<td>37.7±1.5</td>
<td>6.8±0.3</td>
<td>14.3±0.7</td>
<td>0.9±0.1</td>
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<tr>
<td>MSMVs</td>
<td>3.4±0.8</td>
<td>0.4±0.1</td>
<td>7.6±0.1</td>
<td>1.4±0.0</td>
<td>94.0±2.3</td>
<td>6.0±0.3</td>
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<tr>
<td>Ileum homogenate</td>
<td>3.4±0.3</td>
<td>1.0±0.1</td>
<td>5.4±0.6</td>
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<td>15.8±0.8</td>
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<tr>
<td>BLMVs</td>
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<td>10.6±1.1</td>
<td>7.2±0.2</td>
<td>1.4±0.1</td>
<td>14.8±0.5</td>
<td>0.9±0.1</td>
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<td>BBMVs</td>
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<td>40.6±1.4</td>
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<td>1.4±0.1</td>
<td>102±12</td>
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<td>Colon homogenate</td>
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<td>BLMVs</td>
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<td>13.5±2.6</td>
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<td>1.5±0.4</td>
<td>15.1±0.3</td>
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<tr>
<td>BBMVs</td>
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Results are means ± SE of 3 determinations. The activity of Na+-K+-ATPase, nucleotide pyrophosphatase, and NADPH-cytochrome c reductase was determined in homogenate and isolated membrane vesicles from jejunum, ileum, and colon. Enrichment of marker enzymes is also shown. Activity of Na+-K+-ATPase is given as μmol of released Pi/h/mg protein⁻¹. Activity of nucleotide pyrophosphatase is given as μmol of released PNP-h⁻¹/mg protein⁻¹. Activity of NADPH-cytochrome c reductase is given as μmol/h⁻¹/mg protein⁻¹. BLMVs, basolateral membrane vesicles; BBMVs, brush-border membrane vesicles; MSMVs, microsomal membrane vesicles.
0.1 mM PMSF, 5 mg/ml leupeptin, 1 mg/ml pepstatin, and 5 mg/ml aprotinin (pH 7.4, 4°C) and then homogenized 30 times in a Dounce B homogenizer. The cell homogenate was diluted to 40 times its original volume with hypotonic buffer and left with stirring at 4°C for 2 h to destroy the enterocytes completely. The specimen was centrifuged with the discontinuous sorbitol gradient (60, 55, 50, 40, 30, 20, and 10%). The BLMV's fraction was collected from the 30/40% sorbitol interfaces. The brush-border membranes (BBMVs) and microsomal membrane vesicles (MSMVs) were collected from the 40/50% interface and the 50% sorbitol fraction and further purified following Mg\(^{2+}\) precipitation of heavy microsomes. Membrane vesicles were frozen in liquid nitrogen and then transferred to a freezer (−100°C) until required. Activity of Na\(^{+}\)-K\(^{+}\)-ATPase, nucleotide pyrophosphatase, and NADPH-cytochrome c reductase was determined according to the method described by Mircheff et al. (26), Bohme et al. (5), and Phillips and Langdon (31), respectively. The fractions of sealed vesicles and inside-out-oriented membrane vesicles from the three regions were determined by detergent activation of Na\(^{+}\)-K\(^{+}\)-ATPase activity (6).

**Transport studies.** Transport studies were performed using the rapid filtration technique. Briefly, 16 µl transport medium (in mM: 10 Tris-HCl, 250 sucrose, and 10 MgCl\(_2\), pH 7.4) containing radiolabeled compounds, with or without unlabeled substrates, was preincubated at 37°C for 3 min and then rapidly mixed with 4 µl membrane vesicle suspension (8–10 µg protein). The reaction mixture contained 5 mM ATP or 5 mM AMP and ATP-regenerating system (10 mM creatine phosphate and 100 µg/ml creatine phosphokinase). In some instances, the membrane vesicles were treated with acivicin (final concentration 6 mM) at 25°C for 15 min before initiation of the transport studies to avoid possible degradation of LTC\(_4\) by γ-glutamyltranspeptidase. The transport reaction was stopped by the addition of 1 ml ice-cold buffer containing 250 mM sucrose, 0.1 M NaCl, and 10 mM Tris-HCl (pH 7.4). The stopped reaction mixture was filtered through a 0.45-µm membrane filter (GVWP; Millipore, Bedford, MA) and then washed twice with 5 µl stop solution. Radioactivity retained on the filter was determined using a liquid-scintillation counter (LSC-3500, Aloka, Tokyo, Japan).

**Western blot analysis.** Membrane vesicles and homogenate (10 µg protein) were loaded on a 0.75% polyacrylamide slab gel containing 0.1% SDS and then transferred onto a nitrocellulose filter by electroblotting. The filter was blocked with Tris-buffered saline containing 0.05% Tween-20 and 5% BSA for 2 h at room temperature and probed overnight at 4°C with polyclonal anti-Mrp3 antibody (dilution 1:1,000). Antibody was visualized with 125I-labeled anti-rabbit antibody (Amersham Pharmacia Biotech), exposed to Fuji imaging plates (Fuji Photo Film, Kanagawa, Japan) for 3 h at room temperature, and analyzed with an imaging analyzer (BAS 2000, Fuji Photo Film).

**RESULTS**

**Characterization of BL MVs.** The enrichment of marker enzymes was examined in the BL MVs from rat jejunum, ileum, and colon. As shown in Table 1, Na\(^{+}\)-K\(^{+}\)-ATPase, a marker enzyme for the basolateral membrane, was enriched 4.1-, 10.6-, and 13.5-fold in the BL MVs from jejunum, ileum, and colon, respectively, compared with the homogenate. In contrast, no enrichment of nucleotide pyrophosphatase and NADPH-cytochrome c reductase, marker enzymes for the brush-border and microsomal membranes, respectively, was detected in BL MVs (Table 1), although these two enzymes were enriched in the BB MVs and MSMVs, respectively (Table 1). The fractions of sealed- and inside-out-oriented vesicles from the jejunum, ileum, and colon were found to be 29 and 60%, 24 and 45%, and 18 and 62%, respectively, and there was not such a marked difference in the tightness and sidedness of membrane vesicles from the intestinal segments.

**ATP-dependent transport of [\(^{3}\)H]E\(_2\)17βG in BL MVs.** We examined whether organic anions are transported in an ATP-dependent manner. As shown in Fig. 1, [\(^{3}\)H]E\(_2\)17βG was taken up by BL MVs in an ATP-dependent manner. The amount of [\(^{3}\)H]E\(_2\)17βG taken up by BL MVs in an ATP-dependent manner was 34, 67, and 400 pmol/mg protein at 10 min for jejunum, ileum, and colon, respectively (Fig. 1). In contrast to BL MVs, no ATP-dependent uptake of [\(^{3}\)H]E\(_2\)17βG was observed in BB MVs or MSMVs from each part of the rat small intestine (Fig. 2).
Osmotic sensitivity of the uptake of ATP-dependent transport of \([^{3}H]E_{217}B_{G}\) in BLMVs. Osmotic sensitivity was studied by examining the ATP-dependent transport of \([^{3}H]E_{217}B_{G}\) by BLMVs 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 and not by binding to the vesicle surface. The uptake was reduced as the sucrose concentration in the medium increased (Fig. 3). The y-intercept for the relationship between the amount of \([^{3}H]E_{217}B_{G}\) associated with the vesicles vs. the reciprocal of the sucrose concentration in the medium was \(\sim 13\%\) of the total vesicle uptake, if the transport experiment was performed in isotonic medium.

Concentration-dependence of the ATP-dependent uptake of \([^{3}H]E_{217}B_{G}\) by BLMVs. \([^{3}H]E_{217}B_{G}\) was taken up by BLMVs in a concentration-dependent manner (Fig. 4). The ATP-dependent uptake of \([^{3}H]E_{217}B_{G}\) could be described by a single component with a \(K_{m}\) value of 23.5 \(\pm\) 2.8 and 8.31 \(\pm\) 0.58 \(\mu\)M for BLMVs from jejunum and ileum, respectively (Fig. 4). In contrast, the uptake of \([^{3}H]E_{217}B_{G}\) into colon BLMVs could be described by assuming the presence of high \((K_{m} = 0.82 \pm 0.18 \mu\text{M})\) and low \((K_{m} = 35.4 \pm 4.0 \mu\text{M})\)-affinity components (Fig. 4).

Characterization of ATP-dependent transport in BLMVs. The substrate specificity of the ATP-dependent transport located on BLMVs was further characterized. It was found that \([^{3}H]\text{taurocholate}\) was also transported in an ATP-dependent manner (Fig. 5). The amount of \([^{3}H]\text{taurocholate}\) taken up by BLMVs in an ATP-dependent manner was 6, 11, and 110 pmol/mg protein at 10 min for jejunum, ileum, and colon, respectively (Fig. 5). Moreover, it was found that \([^{3}H]\text{TLC-S}\) and \([^{14}C]E_{3040}\) glucuronide were taken up by BLMVs in an ATP-dependent manner (Fig. 6). The extent of the uptake was significantly higher in colon BLMVs compared with jejunum and ileum BLMVs for \([^{3}H]\text{taurocholate}\), \([^{3}H]\text{TLC-S}\), and \([^{14}C]E_{3040}\) glucuronide (Figs. 4 and 5). In contrast, no signif-
The inhibitor sensitivity of the ATP-dependent uptake of \textsuperscript{[3}H\textsubscript{]}E\textsubscript{2}17\textsubscript{B}G into jejunum, ileum, and colon BLMVs was also examined (Fig. 7). The IC\textsubscript{50} values for E\textsubscript{3}040 glucuronide, 4MU glucuronide, \textalpha{}-naphthyl-\textbeta{}-n-glucuronide, DNP-SG, LTC\textsubscript{4}, TLC-S, and taurocholate were 3–10, 30–100, 30–100, 100–300, 10–20, 10–30, and \textminus{}100 \textmu{}M, respectively, for BLMVs from all parts of the intestine (Fig. 7; Table 2). In contrast, 4MU sulfate stimulated the ATP-dependent uptake of E\textsubscript{2}17\textsubscript{B}G into BLMVs (Fig. 7; Table 2).

Expression of Mrp3 in BLMVs. The expression level of Mrp3 was compared in jejunum, ileum, and colon BLMVs based on Western blot analysis. As shown in Fig. 8, bands responsible for Mrp3 were observed in jejunum, ileum, and colon BLMVs but not in their respective homogenates. The band length was \textsim{}190 kDa, which is consistent with that observed for the membrane vesicles isolated from Mrp3 cDNA-transfected LLC-PK1 cells (Fig. 8). Quantitative analysis of the band intensity revealed that the expression of Mrp3 in ileum BLMVs was 28 and 39\% that in colon BLMVs.

DISCUSSION

In the present study, we found that many organic anions are taken up in an ATP-dependent manner by BLMVs from rat intestine. We have to reject the possibility that the observed ATP-dependent uptake is accounted for by contamination of the prepared BLMVs by other membrane fractions. As shown in Table 1, in BLMVs prepared in the present study, the marker enzyme for the basolateral membrane, but not those for the brush-border or microsomal membrane, was significantly enriched. Moreover, we found that the ATP-dependent uptake of \textsuperscript{[3}H\textsubscript{]}E\textsubscript{2}17\textsubscript{B}G is observed in BLMVs but not in BBMVs or MSMVs (Fig. 2). On the basis of the osmotic sensitivity of the uptake by BLMVs, the major part of the radioactivity associated with vesicle specimens is ascribed to uptake and not to adsorption to the vesicles (Fig. 3). These results suggest that the ATP-dependent uptake observed in the present study represents transport across the basolateral membrane. Although Mrp2, which also accepts E\textsubscript{2}17\textsubscript{B}G as a substrate (20, 36, 38), is expressed on the brush-border membrane of enterocytes (28, 32), no ATP-dependent transport of \textsuperscript{[3}H\textsubscript{]}E\textsubscript{2}17\textsubscript{B}G was observed in BBMVs (Fig. 2). This result may be accounted for by the fact that almost all BBMVs from the intestine are composed of right-side-out membrane vesicles (17), and we cannot determine the cellular extrusion of substrate compounds across the brush-border membrane by using BBMVs.

The transporter molecules responsible for the ATP-dependent uptake of organic anions into BLMVs also need to be discussed. Several pieces of evidence are consistent with the hypothesis that Mrp3, located on the intestinal basolateral membrane (32), is at least partly involved in the uptake by BLMVs. First, the substrate specificity of the ATP-dependent transporter in BLMVs resembles that of rat Mrp3. By using membrane vesicles isolated from rat Mrp3-transfected cells, we have previously reported that Mrp3 preferentially accepts glucuronide conjugates such as E\textsubscript{2}17\textsubscript{B}G and E\textsubscript{3}040 glucuronide and sulfated and monovalent bile acids rather than gutathione-conjugates, which is consistent with the present observations in BLMVs (Figs. 1, 5, and 6) (2, 15, 16).

Second, the transport kinetics of E\textsubscript{2}17\textsubscript{B}G in BLMVs are similar to those of recombinant rat Mrp3. The K\textsubscript{m} value of the uptake of E\textsubscript{2}17\textsubscript{B}G was determined as 24 and 8.3 \mu{}M for the jejunum and ileum BLMVs (Fig. 4), which is in the same range as the K\textsubscript{m} value (30 \mu{}M) of recombinant Mrp3 expressed in S9 cells, although the corresponding values in the Mrp3 cDNA-transfected HeLa and LLC-PK1 cells were higher than these values (60–110 \mu{}M) (2, 15, 16). Although the high-affinity component has also been observed for colon BLMVs, the K\textsubscript{m} value of the low-affinity component (35 \mu{}M) is also consistent with the K\textsubscript{m} value of recombinant Mrp3 (Fig. 4). Because the recombinant Mrp3 showed one saturable component for the transport of E\textsubscript{2}17\textsubscript{B}G (2, 15), it is possible that some other unidentified transporters are also involved in the basolateral extrusion of organic anions in the colon.

![Fig. 6. Uptake of \textsuperscript{[14}C\textsubscript{]}6-Hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole (E\textsubscript{3}040) glucuronide and \textsuperscript{[3}H\textsubscript{]}Taurolithocholate-3-sulfate (TLC-S) into BLMVs. BLMVs from jejunum, ileum, and colon were incubated for 10 min in medium containing 12.2 \mu{}M \textsuperscript{[14}C\textsubscript{]}E\textsubscript{3}040 glucuronide (A) or 1.0 \mu{}M \textsuperscript{[3}H\textsubscript{]}TLC-S (B) in the presence of 5 mM ATP (open bars) or 5 mM AMP (filled bars). The results (in \mu{}mol/mg protein) by the ligand concentration in the medium (pmol/\mu{}l). *P < 0.05, ***P < 0.01.](http://ajpgi.physiology.org/)

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*Fig. 6. Uptake of \textsuperscript{[14}C\textsubscript{]}6-Hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole (E\textsubscript{3}040) glucuronide and \textsuperscript{[3}H\textsubscript{]}Taurolithocholate-3-sulfate (TLC-S) into BLMVs. BLMVs from jejunum, ileum, and colon were incubated for 10 min in medium containing 12.2 \mu{}M \textsuperscript{[14}C\textsubscript{]}E\textsubscript{3}040 glucuronide (A) or 1.0 \mu{}M \textsuperscript{[3}H\textsubscript{]}TLC-S (B) in the presence of 5 mM ATP (open bars) or 5 mM AMP (filled bars). The results (in \mu{}mol/mg protein) by the ligand concentration in the medium (pmol/\mu{}l). *P < 0.05, ***P < 0.01.*
Third, the transport activity for E$_2$17$eta$G, E3040 glucuronide, taurocholate, and tauroliothocholic acid sulfate among BLMVs was in the rank order, jejunum $<$ ileum $<$ colon (Fig. 2), which agrees with the order of the expression level of Mrp3 determined by Western blot analysis (Fig. 8). Indeed, the MRP3 expression level in the jejunum and ileum BLMVs was 28 and 39%, respectively, that in colon BLMVs (Fig. 8), which is consistent with the fact that the $V_{\text{max}}/K_m$ values for the transport of E$_2$17$eta$G in jejunum and ileum BLMVs were 18 and 34% of the low-affinity component in colon BLMVs (Fig. 4). Although we found that the expression of Mrp3 is two- to threefold higher in colon BLMVs than ileum and jejunum BLMVs, Rost et al. (32) reported that Mrp3 expression is almost the same between ileum and colon in their mucosa preparation. One of the possible hypotheses to account for this discrepancy is to assume that the ileum Mrp3 is susceptible to degradation during the preparation of BLMVs, although this remains to be clarified.

The fourth piece of evidence in support of the hypothesis that Mrp3 may be involved in the ATP-dependent transport in BLMVs is that the effect of inhibitors was also similar for BLMVs and recombinant Mrp3. As shown in Fig. 7, the IC$_{50}$ values for E3040 glucuronide, 4MU glucuronide, -naphtyl-$\beta$-D-glucuronide, DNP-SG, LTC$_4$, and TLC-S in BLMVs from all parts of the intestine vs. those in the recombinant Mrp3 were $3 \sim 10$ vs. $< 5$ M, $30 \sim 100$ vs. $50$ M, $30 \sim 100$ vs. $20 \sim 50$ M, $100 \sim 300$ vs. $50 \sim 100$ M, $10 \sim 20$ vs. $> 2.5$ M, and $10 \sim 30$ vs. $1 \sim 5$ M, respectively (Fig. 7) (2, 15, 16). Thus the IC$_{50}$ values in BLMVs were also similar to those in the recombinant Mrp3, although the complete agreement was not observed between these two experimental systems. For example, the IC$_{50}$ value for taurocholate in BLMVs was 100–300 

![Fig. 7. Effect of compounds on the uptake of [3H]E$_2$17$eta$G into BLMVs. Jejunum (○), ileum (■), and colon (▲) BLMVs were incubated for 10 min in medium containing 70 nM [3H]E$_2$17$eta$G with or without different concentrations of indicated inhibitors in the presence of 5 mM ATP. The results are given as the %control. Each point and vertical bar represents the mean ± SE of triplicate determinations. E3040G, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl)benzothiazole glucuronide; E3040S, E3040 sulfate; 4MUG, 2-methylumbellifellëne glucuronide; 4MUS, 4MU sulfate; TLC-S, tauroliothocholate-3-sulfate; DNP-SG, 2,4-dinitrophenyl-$\beta$-glutathione; LTC$_4$, leukotriene C$_4$.](http://ajpgi.physiology.org/)

*Fig. 7. Effect of compounds on the uptake of [3H]E$_2$17$eta$G into BLMVs. Jejunum (○), ileum (■), and colon (▲) BLMVs were incubated for 10 min in medium containing 70 nM [3H]E$_2$17$eta$G with or without different concentrations of indicated inhibitors in the presence of 5 mM ATP. The results are given as the %control. Each point and vertical bar represents the mean ± SE of triplicate determinations. E3040G, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl)benzothiazole glucuronide; E3040S, E3040 sulfate; 4MUG, 2-methylumbellifellëne glucuronide; 4MUS, 4MU sulfate; TLC-S, tauroliothocholate-3-sulfate; DNP-SG, 2,4-dinitrophenyl-$\beta$-glutathione; LTC$_4$, leukotriene C$_4$.*
Finally, the possible role of ABC transporters in the absorption of bile acids needs to be discussed. Concerning the uptake of bile acids across the brush-border membrane, ASBT has been identified, which is predominantly expressed in the ileum (24). Moreover, because Oatp3, which transports bile acids in an Na\(^+\)-independent manner, is expressed throughout the small intestinal tract, this transporter and/or its human homologs may also be involved in the jejunal uptake of bile acids as reported in guinea pigs, rabbits, and/or humans (3, 4, 34). Because we found significant ABC transport activity in jejunal BBMVs, it is possible that ABC transporters including Mrp3, at least partly, may be involved in the intestinal absorption of bile acids, although we cannot exclude the possibility that some other mechanisms [such as an anion exchange mechanism (43)] are involved in the basolateral transport of bile acids. Because Mrp3 also transports nonconjugated organic anions, including pravastatin (a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor), temocaprilat (an ACE inhibitor), and BQ-123 (an endothelin antagonist) (39), it is also possible that Mrp3 is at least partly involved in the oral absorption of these anionic drugs. Particularly for ACE inhibitors, it is possible that PEPT1, which is predominantly expressed in the ileum (30), and Mrp3 act synergistically for the vectorial transport from lumen to blood circulation. In addition to these anionic drugs, the intracellularly formed glucuronide conjugates of steroids and xenobiotics may also be transferred into the blood circulation via Mrp3, as summarized previously (39). Moreover, the extensive ABC-dependent transport, along with the extensive expression of Mrp3, found in the colon suggests a possible role in the absorption of bile acids in addition to the predominant role of the ileum (25, 27, 33). In perfused human colon, Mekhjian et al. (25) found that several kinds of bile acids such as chenodeoxycholate, deoxycholate, and cholate are also significantly absorbed. Their analysis suggested that the maximum potent ability of colonic absorption of chenodeoxycholate may be 14 g/day, which is higher than the actual intestinal absorption of this bile acid (5–8 g/day) (25). On the basis of these analyses, they have proposed the hypothesis that the colon may be the anatomic reserve for bile acids not absorbed in the small intestine (25).

In conclusion, it was found that many kinds of organic anions including bile acids are transported in an ABC-dependent manner into rat intestinal BBMVs. Much more extensive transport was observed in colon BBMVs than jejunum and ileum BBMVs. The transport properties in BBMVs suggest that there is a contribution by Mrp3. Such an ABC-dependent mechanism may be involved, at least partly, in the intestinal absorption of organic anions.

**GRANTS**

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**Table 2. IC\(_{50}\) values of compounds on the ATP-dependent uptake of \([\text{H}]\text{E}_2\text{17BG}\) into BBMVs from jejunum, ileum, and colon**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC(_{50}) values, (\mu\text{M})</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3040G</td>
<td>(3\times10^{-3})</td>
</tr>
<tr>
<td>E3040S</td>
<td>(&gt;300)</td>
</tr>
<tr>
<td>4MUG</td>
<td>30–100 (a)</td>
</tr>
<tr>
<td>4MUS</td>
<td>Stimulation (b)</td>
</tr>
<tr>
<td>DNP-SG</td>
<td>100–300 (c)</td>
</tr>
<tr>
<td>LTC-(c)</td>
<td>10–20 (d)</td>
</tr>
<tr>
<td>TCL-(c)</td>
<td>(&gt;10)</td>
</tr>
<tr>
<td>taurocholate</td>
<td>(&gt;100)</td>
</tr>
</tbody>
</table>

Data were taken from Fig. 6. j, i, and c represent uptake of BBMVs from jejunum, ileum, and colon, respectively. \([\text{H}]\text{E}_2\text{17BG}, 17\beta\text{-estradiol-17β-glucuronide; E3040G, 6\text{-hydroxy-5,7\text{-dimethyl-2-methylamino-4-(3-pyridyl}}\text{-methyl}benezothiazole glucuronide; 4MUG, 4\text{-methylumbelliferone glucuronide; 4MUS, 4\text{-MU sulfate; TCL-\(c\), taurolithocholate-3-sulfate; DNP-SG, 2,4-dinitrophenyl-S-glutathione.}]

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**Fig. 8.** Expression of multidrug resistance-associated protein (Mrp3) in BBMVs. The expression level of Mrp3 in BBMVs from jejunum, ileum, and colon was compared based on the Western blot analysis. As a control, membrane vesicles isolated from Mrp3 cDNA-transfected LLC-PK1 cells were also used. Membrane vesicles or homogenate (10 \(\mu\text{g}\) protein) were separated on a 7% polyacrylamide gel containing 0.1% SDS. The fractionated proteins were transferred onto a nitrocellulose filter by electrobobbling followed by detection of Mrp3 protein by incubating with polyclonal anti-rat Mrp3 antisemum. Lane 1: vector-transfected LLC-PK1 cells; lane 2: Mrp3 cDNA-transfected LLC-PK1 cells; lane 3: jejunum homogenate; lane 4: jejunum BBMVs; lane 5: ileum homogenate; lane 6: ileum BBMVs; lane 7: colon homogenate; lane 8: colon BBMVs.


