Review

Intestinal Absorption of Drugs Mediated by Drug Transporters: Mechanisms and Regulation

Toshiya Katsura and Ken-ichi Inui

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto, Japan

Summary: The absorption of drugs from the gastrointestinal tract is one of the important determinants for oral bioavailability. Development of in vitro experimental techniques such as isolated membrane vesicles and cell culture systems has allowed us to elucidate the transport mechanisms of various drugs across the plasma membrane. Recent introduction of molecular biological techniques resulted in the successful identification of drug transporters responsible for the intestinal absorption of a wide variety of drugs. Each transporter exhibits its own substrate specificity, though it usually shows broad substrate specificity. In this review, we first summarize the recent advances in the characterization of drug transporters in the small intestine, classified into peptide transporters, organic cation transporters and organic anion transporters. In particular, peptide transporter (PEPT1) is the best-characterized drug transporter in the small intestine, and therefore its utilization to improve the oral absorption of poorly absorbed drugs is briefly described. In addition, regulation of the activity and expression levels of drug transporters seems to be an important aspect, because alterations in the functional characteristics and/or expression levels of drug transporters in the small intestine could be responsible for the intra- and interindividual variability of oral bioavailability of drugs. As an example, regulation of the activity and expression of PEPT1 is summarized.

Key words: intestinal absorption; transporter; brush-border membrane; basolateral membrane; Caco-2

Introduction

The absorption of drugs from the gastrointestinal tract is one of the important determinants for oral bioavailability. It has long been considered that intestinal absorption of drugs after oral administration is mediated by a simple diffusion process, which depends on physicochemical properties of drugs such as hydrophobicity and ionizing state. However, there have been numerous drugs exhibiting higher absorption rates after oral administration than expected from their physicochemical properties. In the 1980’s, development of in vitro experimental techniques such as isolated membrane vesicles and cell culture systems has allowed us to elucidate the transport mechanisms of various drugs as well as nutrients across the plasma membrane of small intestine and renal proximal tubules. The development of methods isolating the brush-border and basolateral membrane vesicles from these epithelial cells permitted to characterize the detailed mechanisms involved in the transport of various compounds across each membrane. In addition, the human colon adenocarcinoma cell line Caco-2 has been introduced to characterize the transepithelial transport properties of various compounds. This cell line forms confluent monolayers of well-differentiated enterocyte-like cells with functional properties of transporting epithelia and has extensively been used to characterize intestinal transport mechanisms of various drugs. Using isolated intestinal brush-border membrane vesicles or Caco-2 cells, transport characteristics of various drugs have been investigated, and the following criteria were usually used to define the transporter-mediated permeation across the plasma membrane: saturability, temperature-dependence, energy-dependence, cis-inhibition and trans-stimulation effects by related compounds. Modifying reagents for amino acid residues of the membrane proteins were sometimes used to see whether transport process was mediated by transport proteins (transporters). Extensive surveys concerning the intestinal absorption mechanisms for various ionic drugs revealed that drug transporters were mainly classified into three systems; organic cation transport systems, organic anion transport systems and peptide transport systems. However, except for a few drugs, less attention had been paid to transporter-mediated drug absorption in drug discovery and
Table 1. Major transporter families involved in drug absorption and disposition

<table>
<thead>
<tr>
<th>Transporter Family</th>
<th>HGNC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Typical Substrates</th>
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<tbody>
<tr>
<td>ABC transporter</td>
<td>ABCB</td>
<td>hydrophobic compounds, anticancer agents, digoxin, immunosuppressants</td>
</tr>
<tr>
<td>MDR family (MDR1 etc.)</td>
<td></td>
<td>anionic conjugates, anticancer agents, methotrexate, pravastatin</td>
</tr>
<tr>
<td>MRP family (MRP2, MRP3 etc.)</td>
<td>ABCC</td>
<td>di/tripeptides, β-lactam antibiotics, bestatin, valacyclovir</td>
</tr>
<tr>
<td>Peptide transporter PEPT family (PEPT1, PEPT2)</td>
<td>SLC15</td>
<td>lactic acid, salicylic acid</td>
</tr>
<tr>
<td>Monocarboxylic acid transporter MCT family (MCT1 etc.)</td>
<td>SLC16</td>
<td>taurocholic acid, estradiol 17β-glucuronide, sulfobromophthalein, thyroxin, pravastatin</td>
</tr>
<tr>
<td>Organic anion transporter OAT/ oatp family (OATP-C etc.)</td>
<td>SLC21</td>
<td>p-aminohippuric acid, β-lactam antibiotics, estrone-3-sulfate, methotrexate, cimetidine tetraethylammonium, choline, dopamine, 1-methyl-4-phenylpyridinium, cimetidine L-carnitine, tetraethylammonium</td>
</tr>
<tr>
<td>Organic ion transporter OAT family (OAT1, OAT3 etc.)</td>
<td>SLC22</td>
<td>purine/primidine nucleoside, nucleoside derivatives</td>
</tr>
<tr>
<td>OCT family (OCT1, OCT2 etc.)</td>
<td>SLC28</td>
<td>purine/primidine nucleoside, nucleoside derivatives</td>
</tr>
<tr>
<td>OCTN family (OCTN1, OCTN2 etc.)</td>
<td>SLC29</td>
<td>purine/primidine nucleoside, nucleoside derivatives</td>
</tr>
</tbody>
</table>

<sup>a</sup> Gene family nomenclature classified by the Human Gene Nomenclature Committee (HGNC). See its homepage (http://www.gene.ucl.ac.uk/nomenclature/genefamily.shtml) for details.

dvelopment stage and in clinical situations such as drug-drug interactions.

During the last decade, molecular biological techniques have been employed to identify drug transporters responsible for drug absorption from the intestinal lumen. In 1987, Hediger et al.<sup>9</sup> first succeeded in cloning of the Na<sup>+</sup>/glucose cotransporter (SGLT1) from rabbit small intestine by means of a functional expression cloning strategy using Xenopus laevis oocytes.<sup>10</sup> Thereafter, various transporters for nutrients, neurotransmitters and other endogenous compounds have been cloned using this technique, and PCR analyses allowed us to identify homologous genes. Since 1994, when drug transporters such as peptide transporter PEPT1,<sup>11</sup> organic cation transporter OCT1<sup>12</sup> and organic anion transporting polypeptide oatp1<sup>13</sup> were first isolated, many drug transporters have been cloned and characterized. It has been demonstrated that drug transporters are selectively expressed in pharmacokinetically important tissues such as small intestine, liver, kidney and brain capillary endothelial cells. Therefore, it is now well accepted that drug transporters play an important role in drug absorption and disposition. Table 1 summarizes the major transporter families considered to be important determinants for drug absorption and disposition.

In this review, we describe the recent studies concerning the intestinal absorption of drugs mediated by drug transporters. Although the fundamental characteristics of each transporter and the classification of each transporter family (nomenclature) are not described in detail, several excellent reviews covering such information have been published in the last few years.<sup>14–26</sup>

**Transporters Involved in Drug Absorption**

**Peptide Transporters:** Peptide transporters mediate H<sup>+</sup>-coupled active transport of di- or tripeptides across the brush-border membranes of the small intestine and the renal proximal tubules. The acidic luminal pH generated by the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE3) expressed in the brush-border membrane serves as the driving force for the transport of small peptides (Fig. 1). Using intact small intestinal preparations, isolated intestinal brush-border membrane vesicles and Caco-2 cells, it has been demonstrated that intestinal absorption of peptide-like drugs such as orally active β-lactam antibiotics is mediated by the H<sup>+</sup>/peptide cotransport system. In 1994, intestinal peptide transporter was first cloned from rabbit small intestine and designated PEPT1.<sup>11</sup> Thereafter, PEPT1 was cloned and characterized from...
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Fig. 1. Peptide transporters in the small intestine. Di/tripeptides and peptide-like drugs are absorbed across the brush-border membrane (BBM) by H⁺-coupled peptide transporter (PEPT1). Intracellular peptides and peptide-like drugs exit from cells across the basolateral membrane (BLM) by an unidentified peptide transporter.

Due to the broad substrate specificity of PEPT1, it has been considered that utilization of PEPT1 is a promising strategy for oral drug delivery. One successful approach to be a substrate for PEPT1 is peptidyl prodrugs. It was demonstrated to be a substrate for PEPT1 is peptidyl prodrugs. In addition, peptidic prodrugs of a novel aminomethyl tetrahydrofuran-1β-methylcarbapenem (CL 191,121) with L-amino acids such as alanine, valine, isoleucine and phenylalanine have been shown to improve the efficacies after oral administration, though PEPT1-mediated transport was not directly demonstrated. Another possible approach is to conjugate a dipeptide to parental drugs, thus producing peptidyl prodrugs. Ezra et al. synthesized dipeptidyl-bisphosphonates, Pro-Phe-pamidronate and Pro-Phe-alendronate, and successfully demonstrated the improvement of oral absorption of these dipeptidyl-bisphosphonates. After oral administration, intestinal absorption of Pro-Phe-pamidronate and Pro-Phe-alendronate was increased 3-fold. In addition, the apical-to-basolateral transport of these prodrugs across Caco-2 cell monolayers was much greater than that of the parent drug. These in vivo and in vitro studies indicated that PEPT1-mediated transport of dipeptidyl-bisphosphonates was involved in the improvement of oral absorption of bisphosphonates. However, it should be noted that this approach appeared not to be applicable to any compounds. Friedrichsen et al. developed a flexible synthetic pathway for attaching compounds to dipeptides through ester or amide bonds. Glucose-6-phosphatase inhibitor was chosen as a model drug, however, none of the peptide-coupled compounds seemed to be transported by PEPT1 in Caco-2 cells. Since the low aqueous solubility of the parent compounds was not increased after attachment to a dipeptide, they suggested that only compounds with a certain intrinsic aqueous solubility should be targeted to PEPT1 by attachment to a dipeptide. Recently, Nielsen et al. reported that 4-toluenesulfonylureido-carnosine, an anticancer agent synthesized by linking a dipeptide L-carnosine to the parent drug via an amide bond, was not transported by PEPT1 in Caco-2 cells, though it inhibited the PEPT1-mediated uptake of glycyrrhizin.

These strategies utilized dipeptidyl moiety introduced into the parental drug to be recognized by PEPT1. Since endogenous substrates of PEPT1 are di- or tripeptides, it was initially considered that the substrate of PEPT1 should possess a peptide bond and free amino and/or carboxyl terminal in its structure. However, the structure-transport relationships for the interactions of various compounds with PEPT1 revealed that a peptide bond is not an absolute structural requirement to be recognized by PEPT1. Temple et al. first demonstrated that 4-aminophenylacetic acid, a peptide mimic lacking a peptide bond, was transported by PEPT1 and the presence of a peptide bond was not a requirement for rapid translocation. Thereafter, delta-aminolevulinic...
acid, and \( \omega \)-amino fatty acids, all of which lack a peptide bond, were shown to be substrates for PEPT1. Almost at the same time, it was demonstrated that valacyclovir, a prodrug of an antiviral agent acyclovir, interacted with PEPT1 and was directly shown to be a good substrate for PEPT1. Valacyclovir is the L-valyl ester of acyclovir without a peptide bond and has initially been developed to improve the poor oral bioavailability of acyclovir. The finding that transport of valacyclovir is mediated by PEPT1 leads to another idea that 5′-amino acid esterification of poorly absorbed compounds may improve their intestinal absorption. Indeed, transport of valyl esters of AZT and ganciclovir has been shown to be mediated by PEPT1. Sawada et al. demonstrated that L-valyl ester compound showed higher affinity for PEPT1 than other amino acid esters. Taken together, these findings suggest that L-valyl esterification of poorly absorbed drugs may be a useful and promising approach for improving their oral bioavailability.

In addition to the above mentioned approaches to utilize the PEPT1 as the target for oral drug delivery, it should be understood how PEPT1 recognizes its substrate. Bailey et al. provided a model for the orientation of the key binding features of substrates for PEPT1. They showed that this model allowed us to predict the relative affinity of substrates for PEPT1. Although crystal structure of PEPT1 is still not known, a complete understanding of the molecular features recognized by PEPT1 would be important for rational design of drug molecules which can be well absorbed from the small intestine.

Peptide-like drugs taken up by PEPT1 from the intestinal lumen must then exit from the cells across the basolateral membrane of intestinal epithelium. Another peptide transporter localized at the basolateral membrane should mediate this step to achieve the efficient transepithelial transport. The basolateral peptide transporter expressed in Caco-2 cells has been characterized. It was demonstrated that the basolateral peptide transporter is less sensitive to changes in extracellular pH than the apical PEPT1. Glycylsarcosine uptake from the basolateral side was shown to be mediated by a low-affinity, facilitated transport system whereas concentrative uptake of fosinopril was demonstrated. In addition, substrate specificity of the basolateral peptide transporter is somewhat different from that of PEPT1. Most recently, a candidate protein for the basolateral peptide transporter was identified from the rat small intestine. By photoaffinity labeling using [4-azide-3,5-\( \text{H} \)-Phe]-L-Ala as a probe, a single protein band of 112 kDa was identified in the basolateral membrane of rat small intestine. MALDI-TOF analysis of tryptic digests of the protein followed by database searches established that this protein was novel with no obvious similarity to PEPT1. Molecular identification of this protein will allow us to understand the detailed mechanisms of the transport of peptide-like drugs across the basolateral membrane.

**Organic Cation Transporters:** Transport mechanisms of various cationic drugs in the small intestine have been examined using techniques such as everted intestinal sacs, isolated intestinal mucosa mounted on Ussing chambers, isolated brush-border membrane vesicles and Caco-2 cells. Organic cation transport systems characterized earlier were summarized in previous review articles, and we will not discuss previously described transport systems in detail. So far, as compared with the kidney and liver, molecular mechanisms of organic cation transport in the small intestine remain largely to be elucidated. Most organic cation transporters described are not identified yet.

Organic cation transporter 1 (OCT1) is the first member of the OCT family cloned from rat kidney in 1994. Subsequently, members of a family of organic cation transporters (OCT1–3), as well as the more distantly related proteins that transport carnitine and organic cations (OCTN1–3) and organic anion transporters (OAT1–4), have been cloned and characterized. Due to their significant homology, these transporters turned out to be members of the organic ion transporter superfamily (SLC22). Examination of tissue distribution of the OCT family indicated that OCT1 and OCT3 are expressed in the small intestine of rats. OCT1 is expressed much less in the human small intestine, while expression of OCT3 in the human small intestine is not confirmed. Although OCT2 is predominantly expressed in the rat kidney, expression of OCT2 in the human small intestine was detected by RT-PCR. On the analogy of their localization in the renal proximal tubules, localization of OCTs in the small intestine is supposed to be in the basolateral membrane. In addition, recent studies using *Oct1* knockout mouse suggested its basolateral localization in the small intestine (see below). However, direct immunolocalization data are not available so far. Functional characterization of these transporters using various heterologous expression systems revealed that these transporters showed broad substrate specificity. OCTs are likely to function primarily in the elimination of cationic drugs and other xenobiotics in tissues such as kidney, small intestine and liver. However, the role of OCTs in the intestinal transport of cationic drugs is poorly understood.

Recently, Jonker et al. generated mice with a targeted disruption of the organic cation transporter 1 (*Oct1/Slc22a1*) gene. In *Oct1*−/− mice, intestinal secretion of tetraethylammonium was reduced about two-fold after intravenous administration (Table 2), indicating that Oct1 plays an important role in the secretion of organic cations into the small intestinal lumen. In contrast,
the apical-to-basolateral transport, indicating the net secretion of MPP\(^+\). However, addition of tetraethylammonium or decynium-22, inhibitors of OCTs, had no effect on the basolateral-to-apical transport of MPP\(^+\), suggesting the lack of involvement of OCT1 and OCT2 in the secretion of MPP\(^+\). They also indicated the Na\(^+\)-dependent MPP\(^+\) uptake from the apical side of Caco-2 cells grown on permeable supports. Although the nature of this novel Na\(^+\)-dependent transporter is not clear, the results suggest the existence of a novel Na\(^+\)-coupled organic cation transporter in the intestinal brush-border membrane. In contrast, Martel et al.\(^{72}\) have suggested that apical uptake of MPP\(^+\) by Caco-2 cells is mediated by Na\(^+\)-independent transporters, probably OCT1 and OCT3 (they termed EMT as Extraneuronal Monoamine Transporter). However, they evaluated the uptake of MPP\(^+\) by Caco-2 cells grown on plastic dishes and considered the amount of MPP\(^+\) accumulated as the apical uptake. Since the accumulation of substrates by epithelial cells grown on plastic dishes does not necessarily reflect the apical uptake, and OCTs are considered to be located on the basolateral membrane, it seems unlikely that OCT1 and OCT3 mediate the apical uptake of organic cations.

Other organic cation transporters yet to be identified should exist in the small intestine. As mentioned above, transport mechanisms of various cationic drugs have been investigated using a variety of techniques. Mizuuchi et al. examined the transport characteristics of diphenhydramine, an antihistamine, in Caco-2 cells.\(^{70}\) Diphenhydramine uptake by Caco-2 cells was temperature-dependent, saturable and pH-dependent. Chlorpheniramine, another antihistamine, competitively inhibited the diphenhydramine uptake, whereas tetraethylammonium and cimetidine, typical substrates of OCTs, had no effect. In addition, biological amines and neurotransmitters, such as histamine and choline, did not inhibit the diphenhydramine accumulation. These results suggested that transport of diphenhydramine is mediated by a pH-dependent specific transport system in Caco-2 cells. Detailed investigation of the substrate specificity of this transport system indicated the existence of a novel pH-dependent tertiary amine transport system that recognizes N, N-diethyl moieties.\(^{73}\) Although pH-dependent transport of diphenhydramine suggested the involvement of H\(^+\)/organic cation antiport system in diphenhydramine uptake, it is possible that diphenhydramine transport is regulated not by H\(^+\) gradient but by pH itself. Therefore, to determine the driving force of the tertiary amine transport system, the transport of procainamide, another tertiary amine with an N, N-diethyl moiety, by rabbit intestinal brush-border membrane vesicles was examined.\(^{76}\) The data showed that an outward H\(^+\) gradient serves as the driving force for procainamide...
uptake by intestinal brush-border membrane vesicles. This \(H^+\) gradient-dependent transport system specifically recognizes tertiary amines and is different from previously characterized \(H^+/\text{guanidine} \) antiport system\(^{77}\) and \(H^+/\text{thiamine} \) antiport system.\(^{78}\) Taken together, these findings clearly indicate that organic cations with \(N, N\)-dimethyl or \(N, N\)-diethyl moieties such as diphenhydramine and procainamide are transported by a novel \(H^+/\text{tertiary amine} \) antiport system in the intestinal brush-border membrane. Judging from its substrate specificity, it seems likely that characteristics of this transporter are different from those of the OCT family of organic cation transporters. This transport system seems to play an important role in intestinal absorption and/or secretion of tertiary amine compounds.

Other cationic drugs such as azasetron\(^{79}\) and nicotine\(^{80}\) have been reported to be transported by specific transport systems. It was reported that azasetron uptake by Caco-2 cells is saturable, \(Na^+\)-independent, and inhibited by various organic cations such as imipramine, desipramine and serotonin.\(^{79}\) This transport system seemed to be different from the \(Na^+\) and \(Cl^-\)-dependent serotonin transporter. It was demonstrated that transport of nicotine in Caco-2 cells is mediated by a \(pH\)-dependent specific transport system distinct from the \(H^+/\text{tertiary amine} \) antiport system.\(^{80}\) Although the substrate specificity and molecular nature of these transport systems are still unknown, there might be multiple transporters responsible for the absorption of cationic drugs in the small intestine.

**Organic Anion Transporters:** Intestinal absorption mechanisms of anionic drugs have been mainly explained by the passive diffusion of nonionized compounds according to the \(pH\)-partition theory, because intestinal lumen is more acidic than the intracellular \(pH\). However, several studies have suggested the involvement of specific transporters in intestinal absorption of weak acids, especially short-chain fatty acids such as acetic acid and butyric acid (Fig. 2). Monocarboxylic acid transporter (MCT) family, originally cloned from Chinese hamster ovary cells as a lactate transporter MCT1,\(^{81}\) mediates the transport of various short-chain fatty acids.\(^{82}\) Although it was clearly demonstrated that members of MCT family transport various short-chain fatty acids, their role in the intestinal absorption of anionic drugs remains to be elucidated. HMG-CoA reductase inhibitors such as pravastatin and atorvastatin have been suggested to be transported by MCT1, though no direct evidence for MCT1-mediated transport of these drugs has been shown.\(^{83,84}\) Since transport of weak acids such as salicylic acid could be explained not only by a MCT1-mediated mechanism\(^{85}\) but also by a physical process of nonionic diffusion of protonated compound and ion trapping of deprotonated form,\(^{86}\) care must be taken to interpret the data that indicate the pH-dependent, saturable transport of anionic drugs. Direct demonstration of MCT-mediated transport using expression systems is necessary to conclude the MCT-mediated absorption of anionic drugs. Indeed, Okamura et al. recently demonstrated that the uptake of nateglinide, an oral hypoglycemic agent, by Caco-2 cells was saturable, \(pH\)-dependent and inhibited by various monocarboxylic acids.\(^{87}\) Although nateglinide inhibited the MCT1-mediated uptake of L-lactic acid in MCT1-expressing *Xenopus* oocytes, nateglinide itself was not transported by MCT1.\(^{87}\)

Other organic anion transporters have been characterized extensively in the kidney and liver. Organic anion transporters in these tissues are classified into two families: organic anion transporter (OAT) family and organic anion transporting polypeptide (OATP/oatp) family.\(^{15,17,18,20-24}\) OAT1 is the first member of the OAT family identified in 1997.\(^{88}\) and it is almost exclusively expressed in the kidney. So far, members of a family of OAT identified were not expressed in the small intestine, and therefore the role of OAT family members in the intestinal absorption of drugs seems to be negligible. OATP (human)/oatp (rodents) family members are \(Na^+\)-independent, multispecific organic anion transporters that mediate the transport of various amphipathic organic anions as well as conjugated and unconjugated bile salts.\(^{23}\) Among the members of OATP/oatp family, their tissue distribution and substrate specificity is different from each other. The first member of this family, oatp1, was cloned from rat liver in 1994.\(^{13}\) Thereafter, other members were identified from rat, mouse and human. Comparison of tissue distribution and substrate specificity of these members revealed that there were species differences in the expression and function of each transporter.

It was demonstrated that rat oatp3 mRNA is expressed at similar levels down the length of the small intestine.\(^{89}\) Oatp3 is localized to the brush-border membrane of rat jejunum by immunofluorescence and mediates the transport of various bile acids, suggesting that rat oatp3 is the facilitative transporter responsible for the jejunal absorption of bile acids as well as anionic drugs (Fig. 2). It was also demonstrated that the mouse oatp3 gene was localized to a region of chromosome 6 syntenic with human chromosome 12p12, where human OATP-A gene was mapped. Walters et al. therefore suggested that rodent oatp3 is orthologous to human OATP-A.\(^{89}\) However, OATP-A was not expressed in the human small intestine but expressed predominantly in the brain. Tamai et al. examined the expression profiles of human members of the OATP family by RT-PCR and demonstrated the expression of OATP-B, -D and -E in the human small intestine, but not OATP-A and -C.\(^{90}\) Kullak-Ublick et al. reported the expression of OATP-B in the small intestine by Northern blot...
Fig. 3. Mean plasma fexofenadine concentration-time profiles for persons (n = 10) orally administered fexofenadine (120 mg) with 300 mL water, grapefruit juice at 25% of regular strength (25% GFJ), grapefruit juice (GFJ), orange juice (OJ), or apple juice (AJ) followed by 150 mL of the same fluid every 0.5 to 3 hours (total volume, 1.2 L). (From ref. 92)

Fig. 4. ATP-dependent efflux transporters in the small intestine. P-glycoprotein (P-gp), MRP2 and BCRP are expressed in the brush-border membrane (BBM), whereas MRP3 is located at the basolateral membrane (BLM).

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analysis, though OATP-B did not transport bile salts.\textsuperscript{91) Membrane localization of OATPs in the small intestine has not been reported (Fig. 2).

Recently, Kim and colleagues reported that concomitant intake of fruit juices such as grapefruit, orange and apple juices decreased the fexofenadine AUC (Fig. 3).\textsuperscript{92) Since the metabolism of fexofenadine is negligible in human, it seems that such food-drug interaction may occur at the transporter level in the small intestine. Using a recombinant vaccinia virus expression system, they have demonstrated that human OATP-A and rat oatp1–3 mediate fexofenadine uptake.\textsuperscript{92,93) These in vivo and in vitro results suggested that fruit juices inhibited the OATP-mediated fexofenadine absorption, thereby reducing the oral bioavailability. However, as described above, OATP-A is not expressed in the human small intestine. Identification of the human OATP family member(s) responsible for the intestinal absorption of anionic drugs will allow us to understand the food-drug interaction.

Efflux Transporters: It is now well recognized that efflux transporters function as an absorptive barrier and limit the oral bioavailability of various drugs. Efflux transporters include ABC transporters such as P-glycoprotein, breast cancer resistance protein (BCRP) and multidrug resistance associated protein (MRP), and organic ion transporters as described above. Figure 4 shows the localization of ABC transporters expressed in the small intestine. P-glycoprotein and BCRP are expressed in the brush-border membrane and mediate the secretion of various hydrophobic drugs including anticancer agents into the lumen. Recent studies have revealed that expression levels of P-glycoprotein in the human small intestine correlate the absorption profile of several drugs such as talinolol, digoxin and tacrolimus. MRP2 located at the brush-border membrane is considered to mediate the intestinal secretion of anionic drugs as well as conjugated metabolites. In contrast, MRP3 is expressed in the basolateral membrane and reported to mediate the transfer of bile acids to the blood. The role of MRP3 in the intestinal absorption of drugs needs to further be clarified.

Although we should pay attention to efflux transporters to elucidate the oral bioavailability of drugs, we do not describe the detailed characteristics of efflux transporters in this article. Recent reviews focusing on the efflux transporters in the intestinal absorption process have been published elsewhere.\textsuperscript{94–96) Other Transporters: Other nutrient transporters are expressed in the small intestine and have been characterized extensively. Those include Na\textsuperscript{+}/glucose cotransporter, amino acid transporters and nucleoside transporters. Interaction of various drugs with these transporters has been examined, though such studies mainly showed the inhibitory effect of drugs on the transport of typical substrates. Thus, the role of nutrient transporters in the intestinal absorption of drugs needs to be clarified in detail. Among them, nucleoside transporters have relatively been well-characterized and shown to mediate the intestinal absorption of nucleoside derivatives used as anticancer and anti-
**Regulation of Function and Expression of Drug Transporters**

The function and expression levels of drug transporters should be under control of various physiological and exogenous stimuli. Alterations in the functional characteristics and/or expression levels of drug transporters in the small intestine could be responsible for the intra- and interindividual variability of oral bioavailability of drugs often observed in the clinical situations. As already described above, the peptide transporter PEPT1 is the best-characterized transporter in the small intestine in relation to drug absorption. Therefore, we focus on the regulation of PEPT1 activity and expression in the present review. Since a very significant correlation between expression levels of PEPT1 and cephalexin permeability in the rat small intestine was observed (Fig. 5), variation of PEPT1 expression in the small intestine could be correlated with absorption permeability variation of peptide-like drugs. Earlier works concerning the regulation of PEPT1 have been summarized in a previous review. Therefore, updated information is mainly discussed below.

Dietary regulation of peptide transporter in the small intestine had been studied. It was demonstrated that peptide transport activity was increased in response to high protein diets. The mechanism of this response turned out to be increased expression of PEPT1 mRNA and protein. Shiraga et al. reported that supplementation not only with casein or glycyl-phenylalanine but with phenylalanine in the protein-free diet resulted in an increased expression of PEPT1 mRNA in the rat small intestine. In contrast, the expression of PEPT1 protein was decreased when fasted rats were allowed to drink an amino acid mixture. It is still not clear how dietary components such as amino acids and small peptides regulate the expression of PEPT1.

Besides the high protein diets and dipeptide supplementation, the expression of PEPT1 mRNA and protein in the rat small intestine was also increased after a brief fast. The mechanism underlying the up-regulation of PEPT1 expression after a brief fast was considered to be an adaptive response to efficiently absorb small peptides from the intestinal lumen. Ihara et al. also demonstrated that the expression of PEPT1 was increased in rats given total parental nutrition as well as in starved rats. They speculated that insufficient nutritional supply to the whole body might cause an increase in PEPT1 expression. Physiological signals affecting the PEPT1 expression in dietary regulation should further be determined. Recently, Pan et al. demonstrated that the intestinal PEPT1 underwent diurnal regulation in its activity and expression. It is possible that this diurnal rhythm of PEPT1 relates to food content and feeding schedule of rats, because rodents show a nocturnal feeding behavior.

The expression of PEPT1 along with functional properties was also affected by various endogenous and exogenous factors. Increased expression of PEPT1 was demonstrated in Caco-2 cells treated with σ-receptor ligand pentazocine and in streptozotocin-induced diabetic rats. In contrast, several factors were reported to down-regulate the expression of PEPT1. Nielsen et al. reported that treatment of Caco-2 cells with epidermal growth factor resulted in a decrease in PEPT1 expression after long-term treatment. It was also demonstrated that the expression of PEPT1 mRNA and protein was decreased in Caco-2 cells treated with thyroid hormone. Moreover, Shu et al. showed that lipopolysaccharide treatment in rats caused a decreased expression of PEPT1 in the small intestine and this decrease was attenuated by the treatment with dexamethasone. It was suggested that lipopolysaccharide-induced increase in tumor necrosis factor-α and interleukin-1β levels seemed to mediate the decrease in PEPT1 expression. Overall, these factors are likely to regulate, in coordination, the PEPT1 expression that may vary under several disease states. The mechanisms underlying the regulation of PEPT1 expression seemed to be at the transcriptional level and/or post-transcriptional level (i.e. changes in mRNA stability).

Another mechanism of the regulation of PEPT1 after short-term treatment has also been reported. Thamotharan et al. demonstrated that short-term treatment of Caco-2 cells with insulin stimulated the activity...
of PEPT1 without affecting the expression of PEPT1 mRNA. The mechanism was suggested to increase translocation of PEPT1 from a preformed cytoplasmic pool to the apical membrane, because the stimulatory effect of insulin was abolished by colchicine, a microtubule disrupting agent. Subsequently, stimulation of PEPT1 translocation from the intracellular compartment was observed when Caco-2 cells were treated with α2-adrenergic receptor agonists, clonidine and UK14304 or leptin. In both cases, stimulatory effect on PEPT1 was also abolished by colchicine. In addition, leptin treatment resulted in an increased membrane expression of PEPT1 protein and decreased intracellular PEPT1 content, without changes in PEPT1 mRNA level (Fig. 6). However, intracellular compartment where PEPT1 is present is not demonstrated ultrastructurally and signals mediating the translocation of PEPT1 are not known at this stage.

From a pathophysiological point of view, PEPT1 was reported to play an important role in several disease states. Merlin et al. reported that PEPT1 mediated the transport of formyl-Met-Leu-Phe (fMLP), a bacteria-derived major peptide neutrophil chemotactic factor and that PEPT1-mediated fMLP uptake induced neutrophil-transepithelial migration in Caco2-BBE cells, suggesting that PEPT1 mediates intestinal inflammation. They later demonstrated that expression of PEPT1 was observed in the human colon from patients with inflammatory bowel disease such as chronic ulcerative colitis and Crohn’s disease, but not in the normal colon. In addition, it was shown that PEPT1-mediated fMLP uptake enhanced major histocompatibility complex class I surface expression. These data suggested the link between PEPT1 and immune effector status in the epithelial surface of the intestine. Colonic expression of PEPT1 was also found in patients with short-bowel syndrome and this up-regulation of PEPT1 expression was considered to be an adaptive response in patients independent of changes in the mucosal surface area. In contrast to the adaptive response after massive enterectomy, it was reported that midgut resection caused a decreased expression of PEPT1 in rabbit proximal jejunum but had no effect on the expression in the distal ileum. In addition, it was demonstrated that expression of PEPT1 was decreased after allogeneic rat intestinal transplantation even with tacrolimus treatment.

The function of PEPT1 can be altered by modulating its driving force, H+ gradient across the brush-border membrane. Acidic luminal pH is generated by the Na+/H+ exchanger located at the brush-border membrane of intestinal epithelium. It was demonstrated that regulation of NHE3 activity by a specific inhibitor or cAMP indirectly modulates the PEPT1 activity in Caco-2 cells. In addition, Wenzel et al. reported that flavonoids with epidermal growth factor-receptor tyrosine kinase inhibitory activity such as quercetin and genistein enhance the uptake of cefxime, a PEPT1 substrate. It is possible that inhibition of tyrosine kinase by flavonoids activates the apical Na+/H+ exchanger (NHE3), thereby PEPT1 activity is stimulated by maintaining the transmembrane H+ gradient. They also reported that reduction of intracellular free Ca2+ concentration by the treatment of Ca2+ channel blockers such as nifedipine caused an increased uptake of cefxime by Caco-2 cells, whereas cefxime uptake was reduced by increased intracellular free Ca2+ concentration by Ca2+ ionophores. It was already demonstrated that nifedipine administration enhanced the intestinal absorption of β-lactam antibiotics in humans. Wenzel et al. suggested that alterations in intracellular
free Ca\textsuperscript{2+} concentration affected the pH regulatory systems, presumably NHE3, which in turn affected the PEPT1 activity.\textsuperscript{123} Although previous studies have suggested that the effect of nifedipine on the absorption of \beta-lactam antibiotics is mediated by neuronal regulation,\textsuperscript{126,127} Wenzel et al. used Caco-2 cells and therefore excluded the involvement of the enteric nervous system in the regulation of PEPT1 activity by nifedipine.\textsuperscript{123}

**Conclusion**

As summarized above, growing evidence has indicated that numerous drugs are absorbed from the intestinal lumen via various specific transporters. Although the extent of contribution of drug transporters to the overall intestinal absorption process in vivo is not clear in some cases, it is apparent that drug transporters play an important role in the intestinal absorption and are one of the determinants of oral bioavailability. Therefore, molecular identification of yet unknown transporters should be necessary to completely understand the mechanisms of intestinal absorption of various drugs. In addition, the activity and expression levels of drug transporters should be regulated by various endogenous and exogenous factors, for example, hormones, growth factors, diets, drugs, and diseases. Understanding of the mechanisms underlying the regulation of drug transporters will help us to predict the intra- and interindividual variability of oral bioavailability.

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