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Review

Carrier-Mediated Intestinal Transport of Drugs

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Recent advances in the field of carrier-mediated intestinal absorption of of amino acids, oligopeptides, monosaccharides, monocarboxylic acids, phosphate, bile acids and several water-soluble vitamins across brush-border and basolateral membranes are summarized. An understanding of the molecular and functional characteristics of the intestinal membrane transporters will be helpful in the utilization of these transporters for the enhanced oral delivery of poorly absorbed drugs. Some successful examples of the synthesis of prodrugs recognized by the targeted transporters are described. Functional expression of the multidrug resistance gene product, P-glycoprotein, as a primary active transporter in the intestinal brush-border membrane leads to net secretion of some drugs such as anticancer agents in the blood-to-luminal direction, serving as a secretory detoxifying mechanism and as a part of the absorption barrier in the intestine.

KEY WORDS: carrier-mediated transport; transporter; intestinal absorption; amino acid, oligopeptide, glucose; hexose; monocarboxylic acid; lactic acid; short-chain fatty acid; phosphate; bile acid; vitamin; intestinal secretion; active efflux pump; p-glycoprotein; multidrug resistance.

INTRODUCTION

It has long been believed that synthetic drugs are absorbed through the epithelium from the gastrointestinal tract by a simple diffusion mechanism, which would favor lipophilic and unionized drugs. However, there are direct and indirect evidences for participation of carrier-mediated membrane transport mechanisms, where several hydrophilic compounds seem to be absorbed efficiently via such specialized transporters. Therefore, utilization of the intestinal epithelial transporters to facilitate the absorption of appropriately modified drugs seems to be an attractive strategy for improving the bioavailability of poorly absorbed drugs.

This review focuses on the physiological characterization and possible molecular mechanisms of the intestinal brush-border and basolateral membrane transport of various natural compounds (i.e., amino acids, oligopeptides, monosaccharides, inorganic phosphate, monocarboxylic acids, bile acids, and several water-soluble vitamins). We also describe the physiological function of the primary active transporter P-glycoprotein, which is expressed at the brush-border membrane of intestinal epithelium, and acts as a barrier to the intestinal absorption of drugs by producing net basolateral-to-apical flux of xenobiotics. The feasibility of drug absorption, for either parent drugs or appropriately modified drugs, via these transporters is discussed. The transport mechanisms for nutrients and drugs described here are schematically illustrated in Fig. 1.

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SECONDARY ACTIVE TRANSPORT DRIVEN BY Na^+ OR H^+ GRADIENT

Studies with intestinal brush-border membrane vesicles (BBMVs) demonstrate that electrochemical gradients of Na^+ and H^+ play a major role in absorption of glucose, amino acids, bile acids and phosphate (Na^+-gradient dependency) and di/tripeptides, lactic acid, short chain fatty acids and nicotinic acid (H^+-gradient dependency) across the apical membrane of the intestinal epithelial cells (1). An inwardly directed Na^+-gradient can be maintained in living epithelial cells by Na^+, K^+-ATPase present in the basolateral membrane, resulting in an intracellular Na^+ concentration that is lower, by about 10–20 mM, than the extracellular concentration of 100–140 mM (see Fig. 1). Additionally, there is clear evidence for the presence of an H^+-gradient across the intestinal brush-border membrane in the lumen-to-cytoplasm direction. The pH in the close vicinity of the external surface of the brush-border membrane is acidic compared to the pH of the bulk of the luminal fluid. This pH has been determined to be 5.5–6.0 by using microelectrodes in human and in laboratory animals and is known as the "acid microclimate" on the intestinal surface. Since the intracellular pH in the enterocyte is approximately 7.0–7.2, the concentration of H^+ on the luminal side of the brush-border membrane is at least 10 times greater than the cytoplasmic H^+ concentration (2). The Na^+-H^+ exchanger localized in the brush-border membrane is primarily responsible for the intestinal microclimate pH (3). The exchanger catalyzes the entry of Na^+ from the lumen into the enterocytes in exchange for the exit of H^+ from the cell into the lumen.

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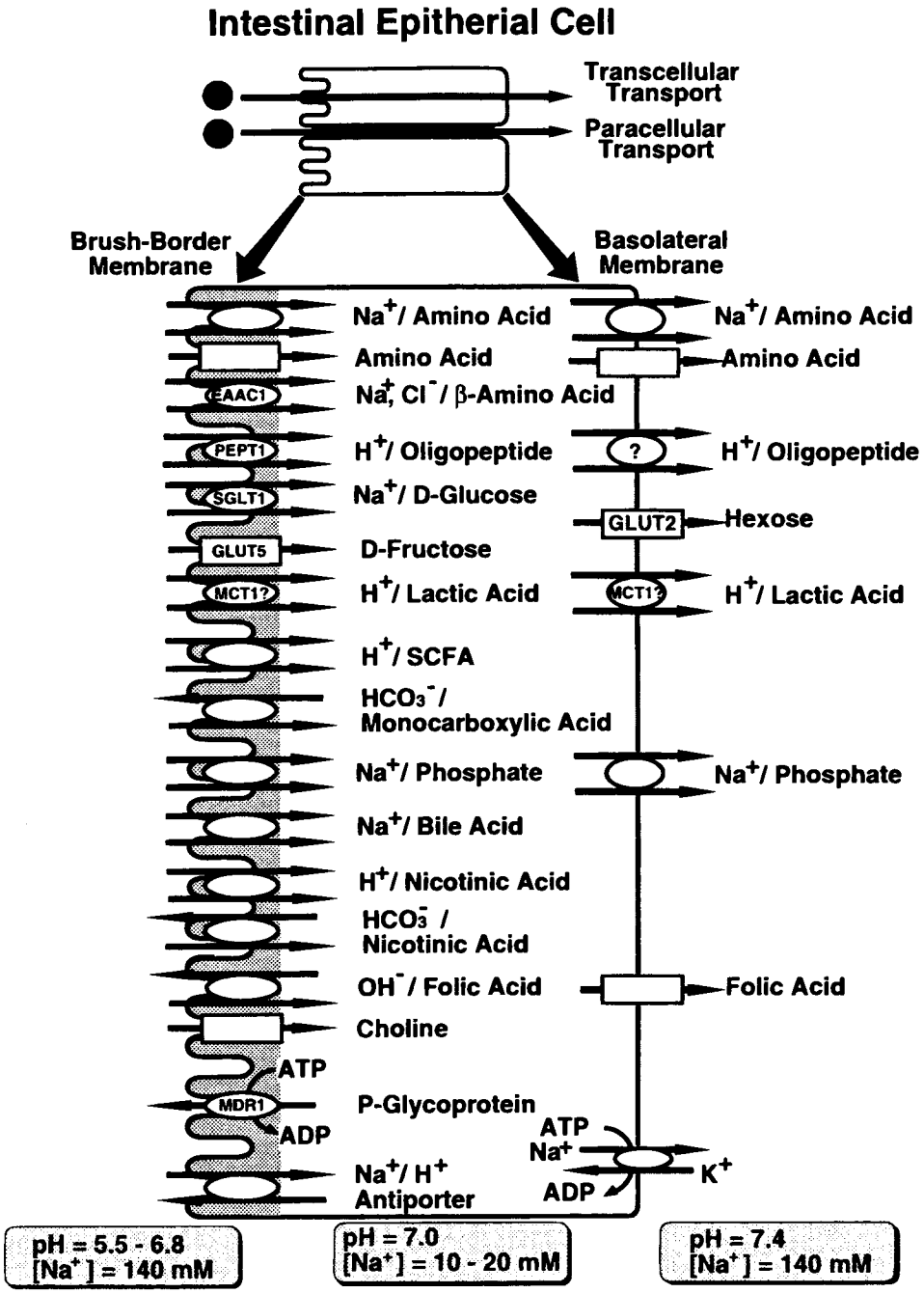

Fig. 1. Summary of intestinal epithelial transporters. Transporters shown by square and oval shapes demonstrate active and facilitated transporters, respectively. The name of cloned transporters were shown within square or oval shapes. In the case of active transporters, the same direction of arrows represent symport of substrate and the driving force. Arrows going in the reverse direction mean the antiport.

TRANSPORT OF AMINO ACIDS AND OLIGOPEPTIDES

Amino Acid Transport

Recently Ganapathy et al. classified amino acid transport systems in the brush-border membrane of small intestine (4). Table I lists their substrate specificity and dependence on ion gradients. However, this classification has not yet been generally accepted. On the other hand, the amino acid transport systems in the intestinal basolateral membrane are classified according to the traditional nomenclature (5) applicable to the plasma membrane of nonpolarized cells (Table II).

System B accepts as substrates nearly all dipolar amino acids that possess the amino group in the α-position. System $B^{0,+}$ is similar to System B, though it accepts not only dipolar amino acids (e.g., leucine), but also basic amino acids (e.g., lysine) as substrates. An inwardly directed Na^+ gradient and an inside-negative membrane potential provide the driving force

Table I. Classification of Amino Acid Transport Systems in the Brush-border Membrane of the Small Intestine (Cited from Ref. 4)

Transport System	Substrates	Dependence on Na^+ Gradient	Involvement of Other Ions
B	Dipolar α-amino acids	Yes	None
$B^{0,+}$	Dipolar α-amino acids Basic amino acids Cystine	Yes	None
$b^{0,+}$	Dipolar α-amino acids Basic amino acids Cystine	No	None
y^+	Basic amino acids	No	None
IMINO	Imino acids	Yes	Cl^-
β	β-Amino acids	Yes	Cl^-
X^-_{AG}	Acidic amino acids	Yes	K^+

for system B and system $B^{0,+}$. The lack of Na^+ dependence is the primary characteristic that distinguishes system $b^{0,+}$ from system $B^{0,+}$. System y^+ transports basic amino acids by an Na^+-independent mechanism and differs from system $b^{0,+}$. The IMINO system accepts exclusively imino acids such as proline, hydroxyproline and pipecolic acid in an Na^+- and Cl^--dependent manner. The β-system accepts nonprotein amino acids, taurine and other β-amino acids and has no affinity for α-amino acids, or acidic/basic amino acids. The IMINO system and the β-system each require both inwardly directed Na^+ and Cl^- gradients as driving forces (6,7). System X_{AG-} accepts acidic amino acids, glutamate and aspartate (4).

Human carcinoma cell line Caco-2 has the Na^+-dependent and -independent amino acid transport systems described above. Caco-2 cells were recently demonstrated to express, at the apical membrane, novel Na^+-independent and H^+-coupled transport systems recognizing β-alanine, L-alanine, proline and α-methylaminoisobutyric acid (8).

The intestinal amino acid transport system, y^+, was the first mammalian amino acid transporter to be successfully cloned. It is a protein with 622 amino acid residues and is predicted to have 14 membrane-spanning domains. Injection of cRNA transcribed from the cloned cDNA into *Xenopus laevis* oocytes leads to an increase in the Na^+-independent transport of cationic amino acids, lysine, arginine and ornithine, but does not induce cysteine transport. The Na^+-independent amino acid transport system, $b^{0,+}$, has been cloned from rat and rabbit kidney, and is also expressed in the small intestine. This transport protein referred to as NBAT, exhibits no homology with system y^+. A cDNA for rabbit intestinal Na^+-dependent glutamate transporter, referred to as EAAC1, was isolated and shown to encode a 524 amino acid protein predicted to have ten membrane-spanning domains (9).

Table II. Classification of Amino Acid Transport Systems in the Basolateral Membrane of the Small Intestine (Cited from Ref. 4)

Transport System	Substrates	Dependence on Na^+ Gradient
A	Dipolar α-amino acids Imino acids	Yes
ASC	Three- and four-carbon dipolar amino acids	Yes
asc	Three- and four-carbon dipolar amino acids	No
L	Bulky, hydrophobic, dipolar amino acids	No
y^+	Basic amino acids	No

Amino Acid-mimetic Drug Absorption via Amino Acid Transporters

Gabapentin (structure in Fig. 2), an analogue of GABA with neuroprotective action and antiepileptic properties, is absorbed slowly following oral administration, with a decreased absorption from 74% to 36% as the gabapentin dose is increased from 100 to 1600 mg. Studies with rat intestinal perfusion and everted rat intestinal rings indicated that gabapentin is absorbed from the small intestine, though not efficiently, by the transporter for large neutral amino acids (10).

Several amino acid analogues (Fig. 2) such as α-methyldopa (11,12), L-dopa (13) and baclofen (14) by large neutral amino acid transporter and D-cycloserin (15) by proton-coupled amino acid transporter, respectively, are also absorbed from the small intestine via each amino acid transport system.

Oligopeptide Transport

Although it has long been believed that intestinal peptide transport can be energized by the Na^+ gradient, H^+ gradient dependence has now been widely accepted, and the phenomenon has been confirmed in various animal species including human and with different tissue preparations. The coupling of intestinal peptide transport to H^+ rather than Na^+ is an important distinction between intestinal peptide transport and amino acid transport (16). As clearly shown in Fig. 3, in the human intestinal brush-border membrane vesicles an inwardly directed H^+ gradient markedly stimulates the transport of the dipeptide glycyl-glutamine but does not affect the transport of the amino acid glutamine (17).

An H^+-coupled peptide transporter, PepT1 was cloned in 1994 from rabbit intestine (18) and in 1995 from human intestine (19). The cDNA encodes 707 and 708 amino acid residues for rabbit and human PepT1, respectively, with twelve putative membrane-spanning regions and an unusually large hydrophilic loop having several N-glycosylation sites (Fig. 4). We have also cloned the homologue of rabbit PepT1 gene from a rat intestinal cDNA library. The cDNA sequence of rat PepT1 is composed of 710 amino acids and the predicted amino acid sequence shows 77% and 83% identity with rabbit and human PepT1, respectively (20).

Studies of complementary RNA (cRNA) of rabbit (18) and human intestinal PepT1 (19) in *Xenopus laevis* oocytes revealed that [^{14}C]glycylsarcosine (gly-sar) transport was enhanced in the presence of an inwardly directed H^+-gradient. Measurements of intracellular pH in oocytes impaled with pH microelectrodes revealed that the peptide transport is associated with intracellular acidification. Stoichiometric studies showed that each gly-sar molecule is co-transported with one H^+, giving a 1:1 stoichiometry. PepT1 is predicted to be able to concentrate neutral oligopeptides up to about 300-fold across brush-border membranes at the extracellular pH of 5.5 (18). Studies on rabbit PepT1-mediated transport by two-microelectrode voltage-clamp analysis of rabbit PepT1 cRNA-injected oocytes revealed that small peptides containing either neutral, basic or acidic amino acids are transport substrates and that peptides larger

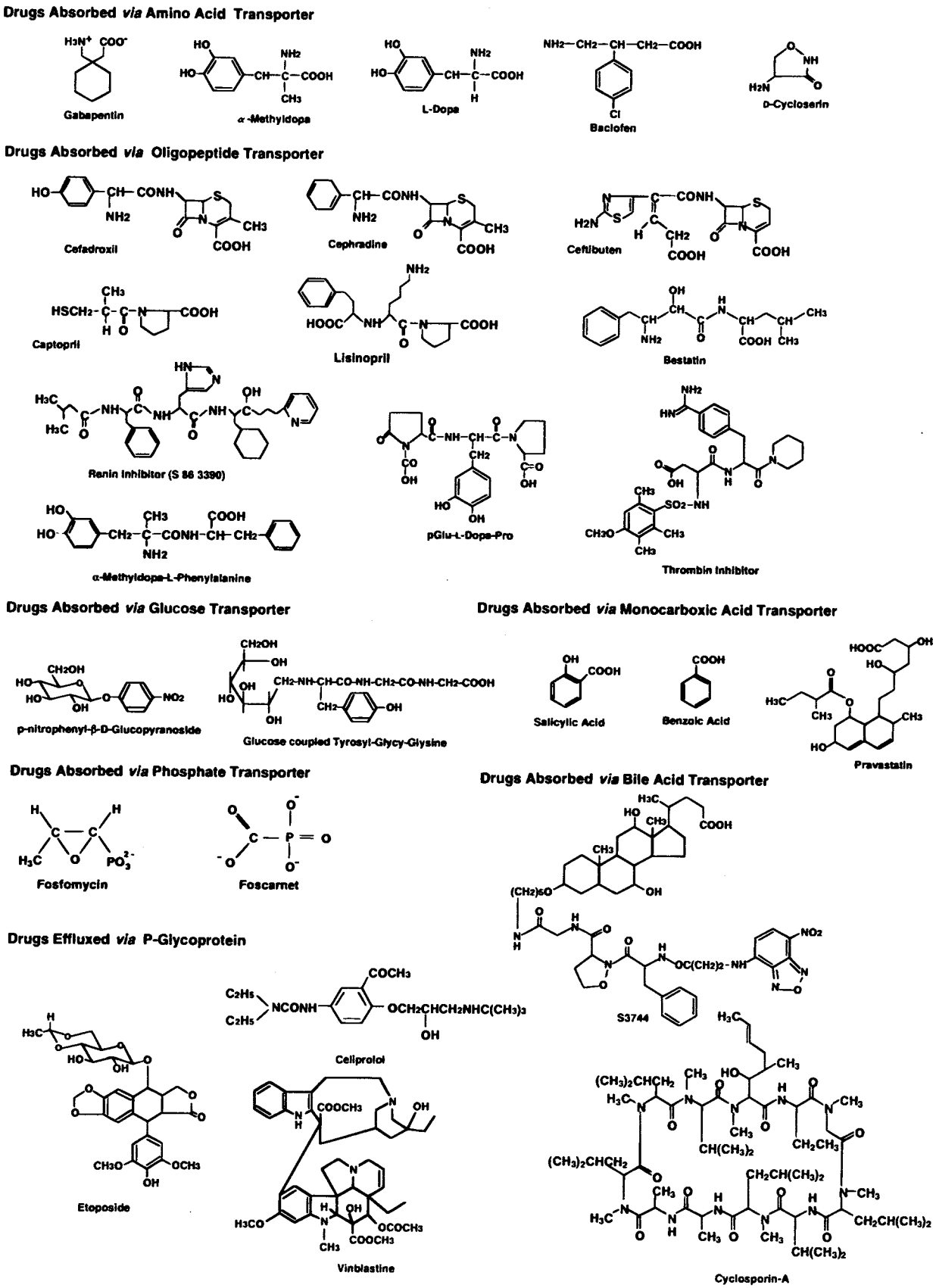

Fig. 2. Classes and chemical structures of drugs which are absorbed via intestinal transporters.

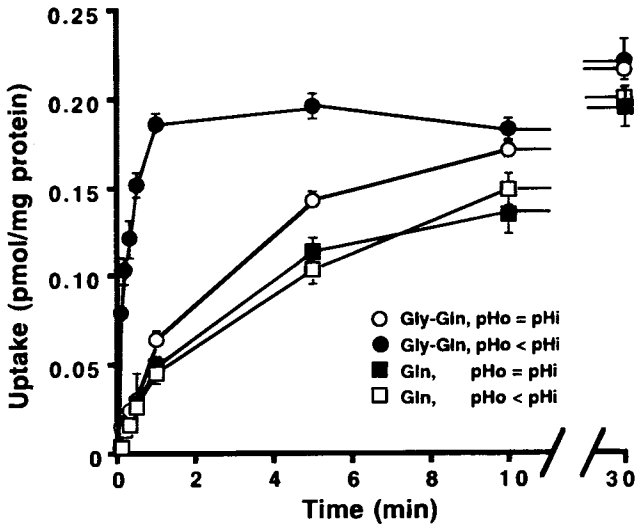

Fig. 3. H^+-dependence of glycyl-glutamine transport studied with purified human intestinal brush-border membrane vesicles. Cited from Ref. 4.

than tetrapeptides are not transported. Similarly, [^{14}C]gly-sar uptake induced with cRNA injection into oocytes of our cloned rat intestinal PepT1 was specifically inhibited by dipeptides and tripeptides, but not by their constituent amino acids or by tetra- or pentapeptides (21).

In contrast to transport across the brush-border membranes, only limited information is available about oligopeptide transport across the basolateral membranes of the intestine. Uptake of [^{14}C]glycyl-L-proline by rabbit proximal intestinal basolateral membrane vesicles oriented inside-out was stimulated in the presence of an inwardly directed pH gradient and followed Michaelis-Menten kinetics with a Km value of 2.0 mM. The uptake was significantly inhibited by 10 mM glycyl-dipeptides and 10 mM cephradine, suggesting the existence of a proton-coupled oligopeptide transporter in the intestinal basolateral membrane similar to that in the brush-border membrane (22). Basolateral membrane of Caco-2 cells possesses an electrogenic H^+-coupled dipeptide transporter, as demonstrated by measuring the transport of [^{14}C]gly-sar and monitoring dipeptide-stimulated H^+-influx across the basolateral membrane (23). The localization of rabbit PepT1 has been confirmed with PepT1 antibody to be limited to the intestinal apical membrane (Tsuji et al., unpublished observation), suggesting the existence of a different H^+/oligopeptide transporter(s) from PepT1 in the intestinal basolateral membrane.

An isoform of PepT1, PepT2, has been recently isolated from human kidney and shown to be ~50% identical and 70% similar to PepT1. Functional expression of the kidney cDNA in HeLa cells resulted in the induction of an H^+-coupled transport specific for di- and tri-peptides and aminocephalosporins. This transporter PepT2 is mostly expressed in the kidney, but not in the small intestine (24).

Absorption of Peptide-mimetic Drugs via Oligopeptide Transporter

Many studies have shown that certain hydrophilic β-lactam antibiotics (structures in Fig. 2) can be transported by oligopeptide transporters in intestinal tissue preparations, isolated intestinal brush-border membrane vesicles, and Caco-2 cells (25). As shown in Fig. 5, a clear overshoot phenomenon was observed for cephradine in rabbit intestinal brush-border membrane vesicles, when an inwardly directed H^+ gradient was imposed (26). Such a stimulated uptake in the presence of an inwardly directed H^+ gradient was reduced significantly when FCCP, a protonophore, was preloaded (Fig. 5, Panel B) or when various dipeptides were added, suggesting that cephradine was taken up by the brush-border membrane H^+/oligopeptide transporter(s). Efflux of cephradine from Caco-2 cells is also via a carrier-mediated process (27), probably via the basolateral H^+/oligopeptide transport system described above.

We have reported the expression of a transporter for both zwitterionic (cefadroxil) and di-anionic (ceftibuten) β-lactam antibiotics in *Xenopus laevis* oocytes injected with mRNA obtained from rat, rabbit and human small intestinal epithelial cells (28,29). The transporter expressed in oocytes was considered to be an H^+/oligopeptide transporter, as judged from the pH-dependence of the transport activity for several β-lactam antibiotics and from the substrate specificity evaluated on the basis of inhibitory and countertransport effects. The transport was stereospecific, i.e., uptake of the *cis*-isomer (ceftibuten) was stimulated in rat intestinal brush-border membrane vesicles in the presence of H^+ gradient, but that of the *trans*-isomer was not in oocytes which expressed an H^+/oligopeptide transporter(s) after injection of rat intestinal mRNA.

Our cloned rat PepT1 exhibited transport activity for β-lactam antibiotics, cephalexin, cephradine, cefadroxil, cefixime, and ceftibuten (*cis*-isomer) but not for cefazolin or the *trans*-isomer of ceftibuten (Fig. 6). The mutual inhibitory effects between dipeptides and β-lactam antibiotics on uptake by rat PepT1 expressed in *Xenopus laevis* oocytes indicate possibly common binding site(s) on the PepT1 (21). Evidence for H^+/cefadroxil transport activity, which was inhibited by cephaloglycine, cefaclor, ampicillin, enalapril, and captopril, but not by cefamandol, cephalothin, benzylpenicillin, or lisinopril at the concentrations of 10 mM, was also observed in oocytes expressing rabbit PepT1 (30). These results show that orally absorbed cephalosporins, aminopenicillins, captopril, and enalapril, but not parenterally used cephalosporins, benzylpenicillin, and lisinopril, are substrates for the H^+/oligopeptide transporter.

Several peptidomimetic drugs (see Fig. 2) other than β-lactam antibiotics, i.e., angiotensin-converting enzyme inhibitors such as captopril, enalapril, lisinopril (30–32), renin inhibitors (33,34), anticancer drugs such as bestatin (35), and peptidomimetic thrombin inhibitors (36), have been proposed to be taken up by the intestinal H^+/oligopeptide transporter.

The intestinal peptide transport system can be employed to improve intestinal absorption of certain drugs by chemically converting them to di- or tripeptide type prodrugs. The very low bioavailability of α-methyldopa, which is taken up by Na^+-coupled neutral amino acid transporter has been improved by the use of dipeptide prodrugs, such as α-methyldopa-L-phenylalanine (Fig. 7), which can be taken up by the intestinal H^+/oligopeptide transporter (31,32,37). To overcome the low bioavailability of oral L-dopa due to decarboxylation in the gut wall, a tripeptide prodrug of L-dopa, p-glu-L-dopa-pro was designed to be absorbed via the intestinal peptide transporter, so as to minimize the decarboxylation in the gut wall, and to be converted to L-dopa by peptidases, with cleavage by

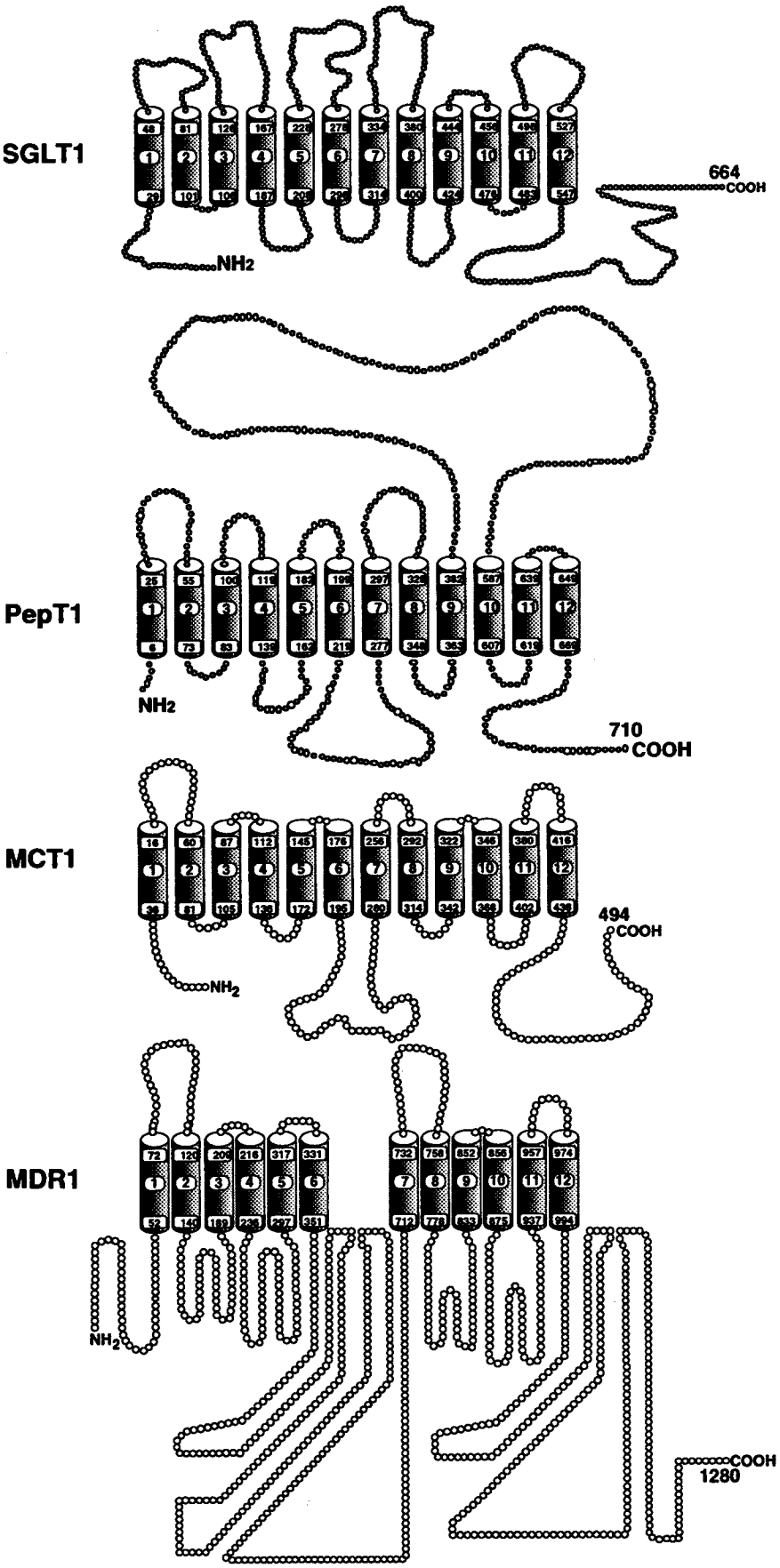

Fig. 4. Structural models of human Na^+/glucose transporter (SGLT1), rabbit H^+/oligopeptide transporter (PepT1), rat H^+/monocarboxylic acid transporter (MCT1) and human MDR1. Cited from Ref. 9, Ref. 65, and Ref 110.

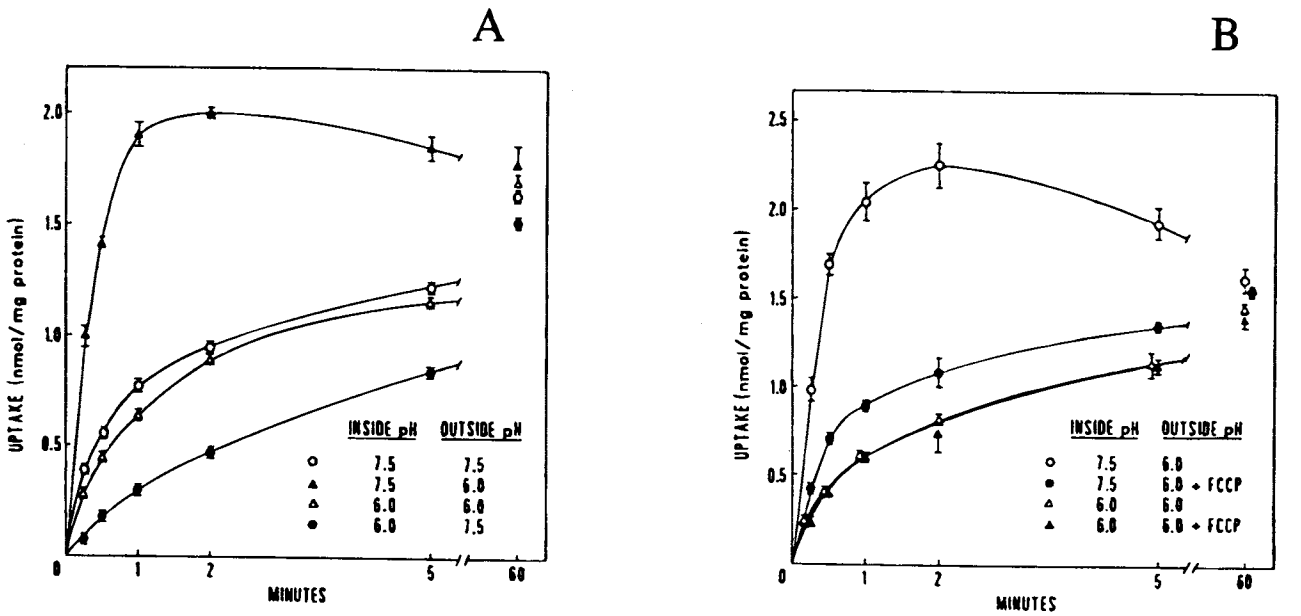

Fig. 5. Effects of outer medium pH (Panel A) and FCCP (Panel B) on cephradine uptake by rabbit intestinal brush-border membrane vesicles. Cited from Ref. 26.

pyroglutamyl aminopeptidase I to L-dopa-pro as the rate-limiting step (38).

Therefore, it is clear that the design of prodrugs suitable for transport by the peptide transporter can be a useful strategy for improving the absorption of small polar drugs which exhibit very poor bioavailability.

CARBOHYDRATE TRANSPORT

Many membrane transport studies on carbohydrates have shown that three mechanisms, active transport, facilitated transport, and passive diffusion/paracellular transport, operate in parallel for the transfer of these hexoses into blood stream. Although several studies have indicated a significant participation of paracellular absorption (39), more studies are needed to clarify the relative importance of saturable and nonsaturable transport mechanisms in the intestinal absorption of monosaccharides under physiological conditions.

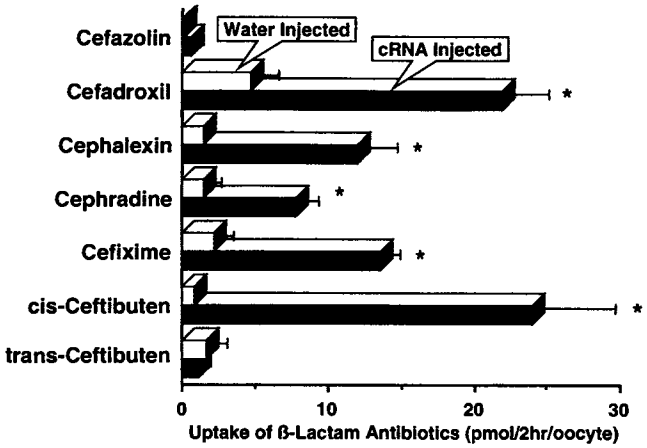

Fig. 6. Uptake of cephalosporins (2 mM) by *Xeopus laevis* oocytes injected with rat intestinal H^+/oligopeptide transporter, PepT1 cRNA (closed column) or water (open column) at 27°C. Cited from Ref. 21.

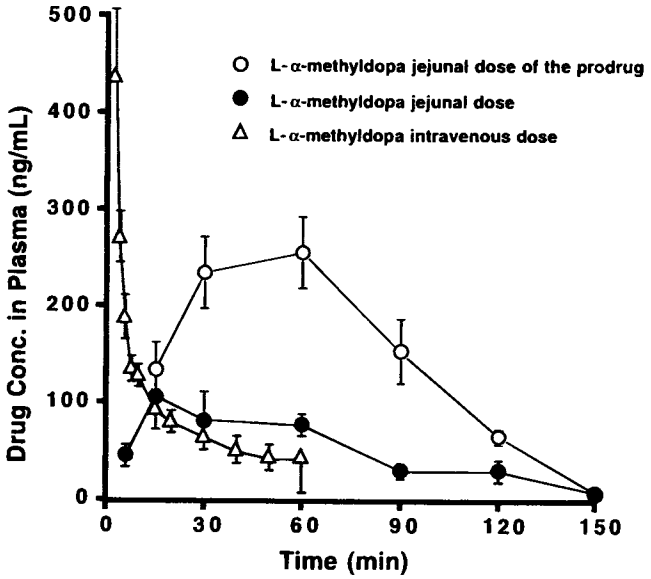

Fig. 7. Plasma profiles of L-α-methyldopa following intravenous dose of L-α-methyldopa and jejunal dose of L-α-methyldopa-phenylalanine and L-α-methyldopa. Cited from Ref. 32.

Sodium Ion-Dependent Active Transport of Monosaccharides

Active transport of D-glucose across the intestinal brush-border is energized by the electrochemical gradient of sodium ions. The sodium ion-D-glucose cotransporter has been cloned by the expression cloning technique using a heterologous gene expression system in *Xenopus laevis* oocytes, and it was named SGLT1 (40). SGLT1 is a hydrophobic integral membrane pro-

tein with approximately twelve putative membrane-spanning domains (Fig. 4). Homologous clones were isolated for SGLT1 from rat, pig and human (41,42).

Facilitated Transport of Monosaccharides

D-Fructose is absorbed slowly but significantly from the intestine. A cDNA clone encoding GLUT5, a candidate for the D-fructose transporter, was isolated from a human jejunal cDNA library (43). D-Fructose transport was sodium-independent and not inhibited by D-glucose, D-galactose, sucrose, or α-methylglucopyranoside. GLUT5 cDNA was also cloned from rabbit and rat small intestines, and the expressed proteins showed functional similarities with human GLUT5 (44,45). From these results, the intestinal brush-border membrane transport of hexoses can be ascribed to a sodium-dependent active transporter SGLT1 for D-glucose, D-galactose, and their analogues and a facilitative transporter GLUT5 for D-fructose.

Basolateral Transport of Monosaccharides

Monosaccharides accumulated in enterocytes are transported by a facilitative transporter across the basolateral membranes into blood. From cDNA libraries of rat liver and human liver, cDNA for the glucose transporter GLUT2 was cloned (46,47). The transporter was suggested to exist in the intestine and to have a 2-deoxy-D-glucose transport activity. Immunofluorescence analysis by using GLUT2 antibodies (48) revealed the presence of the protein only in the intestinal basolateral membrane, not in the brush-border membrane. Accordingly, transport of glucose across intestinal basolateral membranes appears to be mediated by the facilitative transporter GLUT2. Intestinal epithelial transport of monosaccharides has been well reviewed (9).

Utilization of Monosaccharide Transporters for Drug Absorption

There have been several attempts to facilitate intestinal absorption and tissue distribution of less permeable compounds by utilizing monosaccharide transport systems, through modification of parent compounds to sugar analogues. Permeation of *p*-nitrophenyl β-D-glucopyranoside (Fig. 2) across rat everted jejunum was comparable with that of D-glucose and was significantly reduced in the presence of phlorizin and by replacement of sodium ions in the incubation medium with potassium ions (49). Interestingly, permeation of *p*-nitrophenyl β-D-galactopyranoside was lower than that of glucose conjugates (50), which is consistent with the affinity of monosaccharides for SGLT1; namely, glucose has a higher affinity than galactose.

A strategy for the enhancement of intestinal absorption by derivatization to monosaccharide analogues was also applied to peptides. Mono- or disaccharide derivatives of tyrosyl-glycyl-glycine appeared on the serosal side and no metabolites of them were detected following the addition of these compounds to the mucosa of intestines. Although the improved intestinal absorption has not yet been definitively ascribed to the intestinal sugar transporters, the coupling of unstable peptides with sugars does improve both hydrolytic stability and membrane permeation (51). A much larger peptide, insulin was also modified with *p*-nitrophenyl-α-D-gluco-pyranoside and *p*-nitrophenyl-α-D-mannopyranoside, where the expected hypoglycemic effects were observed after intra-intestinal administration in rats. In contrast, the *p*-nitrophenyl-α-L-arabino-pyranoside-insulin derivative was not effective. Intestinal absorption of insulin apparently occurred following modification of insulin with sugar, and may reflect both increased resistance to enzymatic hydrolysis and enhanced membrane permeation (52).

MONOCARBOXYLIC ACID TRANSPORT

Passive Diffusion by pH-Partition and pH-Dependent Carrier-Mediated Transport

Apparent increase in the intestinal absorption of weak organic acids with decreased pH has been empirically explained by pH-partition theory, by assuming that the un-ionized form of the acid permeates passively through the intestinal epithelial membranes. However, a significant shift of apparent pKa from the true value to a more alkaline pH, as evaluated from the pH-absorption rate profile, is often observed in the intestinal absorption of weak organic acids, resulting in a greater absorption than expected from the theory (53). Several modifications of the theory have been proposed, including participation of paracellular transport of drugs, the presence of a mucosal unstirred water layer and the importance of an acidic microclimate (54). All of these modifications of pH-partition theory retain the assumption that organic weak acids are absorbed by passive diffusion. Although intestinal absorption by passive diffusion definitely occurs, there are several studies which suggest involvement of carrier-mediated transport across intestinal epithelial cells, mainly brush-border membrane, for several natural and synthetic weak organic acids, as described below.

Transport of Lactic Acid and Short-chain Fatty Acids

Lactic acid (pKa 3.86) largely exists as a dissociated form at the pH of intestinal luminal solution and is expected to be transported across plasma membranes by some specialized mechanism, but not by passive diffusion. A predominant role of proton gradient-dependent transport for lactic acid and its analogues over sodium-dependent transport was reported in intestinal brush-border membrane vesicles of humans (55).

Short-chain fatty acids (SCFA), which are also called volatile fatty acids (including acetic acid, propionic acid and butyric acid), are produced in the gut by microbial digestion of carbohydrates. The intestinal absorption of SCFA is now ascribed to both non-ionic passive diffusion and carrier-mediated transport (56). As a pH-dependent carrier-mediated absorption mechanism for SCFA, a proton-cotransport system was postulated to function in rabbit intestinal brush-border membrane vesicles (57). In that study, initial uptake of [^{3}H]acetic acid (4.5 μM) by the brush-border membrane vesicles increased with decreased pH from 7.5 to 5.0, as shown in Fig. 8. The uptake was completely abolished in the presence of an excess amount of unlabeled acetic acid (500 μM). The initial uptake of [^{3}H]acetic acid by protein-free liposomes made from egg yolk lecithin was also increased at acidic pH. However, the addition of unlabeled acetic acid did not affect the uptake by the liposomes. Accordingly, the decrease in the uptake of [^{3}H]acetic acid by the intestinal brush-border membrane vesicles in the presence of unlabeled acetic acid is considered to be due to a specific carrier-mediated transport mechanism, presumably a proton-cotransport mechanism, but not via passive diffusion.

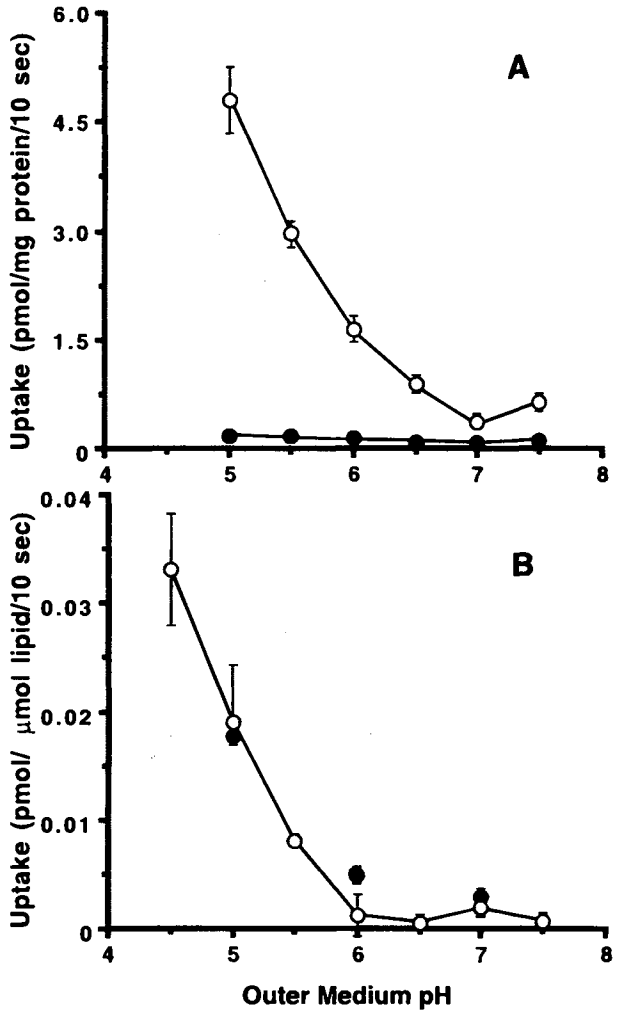

Fig. 8. Effect of outer medium pH on the initial uptake of [^{3}H]acetic acid by rabbit intestinal brush-border membrane vesicles (A) and by egg yolk liposomes (B). The initial uptake rates of [^{3}H]acetic acid were determined at 10 sec at 27°C in the presence (closed circles) or absence (open circles) of an excess amount of unlabeled acetic acid (0.5 mM). The concentrations of [^{3}H]acetic acid used were 4.5 μM for the membrane vesicle study (A) and 8.3 μM for the liposome study (B). In intestinal brush-border membrane vesicles, [^{3}H]acetic acid uptake was significantly reduced in the presence of unlabeled acetic acid, whereas no reduction was observed in the uptake by liposomes. Cited from Ref. 57.

That the SCFA influx is coupled with an efflux of bicarbonate was suggested in rat and human colon by the observation of an enhanced alkalinization in intestinal luminal fluid by SCFA, using an Ussing-type chamber (58). We also observed similar enhancement of luminal alkalinization upon addition of acetic acid to the luminal bathing solution using rabbit small intestinal segment (unpublished observation in our laboratory). Anion exchanger-mediated transport of acetic acid was also demonstrated in the intestinal brush-border and basolateral membrane vesicles from herbivorous teleost (59) and from rabbit (60) and similar transport of propionic acid was found for humans (61). Interestingly, in those studies, such exchange transport of SCFA with bicarbonate was apparently enhanced at acidic extravesicular pH. Anion exchanger-mediated transport of SCFA is likely to be affected by microclimate pH at the intestinal membrane surface in vivo.

Recently, the rabbit erythrocyte lactic acid transporter was purified and a partial N-terminal amino acid sequence was obtained (62). The molecular size of the transporter is 40–50 kilodaltons and the N-terminal sequence was PPAVGGPV-GYTPPDGG. Interestingly, this sequence is identical to the predicted N-terminal sequence of the protein encoded by cDNA for the proton-coupled monocarboxylic acid transporter, MCT1, cloned from Chinese hamster ovary (CHO) cells (63). By northern blot hybridization using CHO MCT1 as the probe, we identified MCT1 in rat and rabbit intestines and in Caco-2 cells (64). The size of mRNA in rats and rabbits hybridized with CHO-MCT1 was about 3.4 kilobases, which is consistent with that found in CHO cells. We screened a rat intestinal cDNA library by using CHO-MCT1 as the probe and obtained the rat MCT1 cDNA (3.4–3.6 kilobases) (Fig. 4). The cDNA was inserted into pBluescript SK(−) and the cRNA was synthesized. When cRNA encoding rat MCT1 was injected into *Xenopus laevis* oocytes, stereospecific and pH-dependent transport activities for lactic acid (65), as well as for SCFA such as acetic acid and pyruvic acid were observed (Tsuji et al., unpublished observation). Thus, it is highly likely that MCT1 cloned from the rat intestine plays a role in the transport of lactic acid, SCFA, and other monocarboxylic acids in the small intestine.

Transport of Monocarboxylic Acid-type Drugs

Many synthetic monocarboxylic acid compounds have been thought to be absorbed mainly by passive diffusion according to the pH-partition theory. In this section, several lines of evidence are presented to suggest participation of carrier-mediated transport mechanisms for monocarboxylic acid drugs.

Intestinal absorption of salicylic acid has been studied in detail, which suggest both specific carrier-mediated transport and passive diffusion participate in salicylate transport (53,66). Transcellular transport of salicylic acid and benzoic acid across Caco-2 cells showed several characteristics of carrier-mediated transport mechanisms (67,68). In salicylic acid transport, saturability and *cis*-inhibitory effects by structural analogues were observed. Interestingly, a fairly good correlation was observed between intestinal absorption rate constants obtained by the *in situ* rat intestinal loop method and apparent affinity for the putative transporter evaluated from the inhibitory potencies of several salicylic acid analogues (67). All of these observations suggest that a carrier-mediated transport mechanism is important for the transport of benzoic acid and salicylic acid in Caco-2, and a similar mechanism may function in intestinal absorption.

Pravastatin, a water-soluble 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is classified as a monocarboxylic acid and has a pKa of 4.7. Its bioavailability after oral administration is fairly high, and other monocarboxylic acid-type HMG-CoA reductase inhibitors also show good bioavailability, comparable with those of lipid-soluble prodrug (lactone) analogues. Uptake of pravastatin by brush-border membrane vesicles was increased at acidic pH and showed an overshoot in the presence of an inwardly-directed proton gradient. The uptake rate estimated for 10 sec was saturable with a Km of 15 mM at an extravesicular pH of 5.5. Pravastatin uptake was reduced in the presence of several monocarboxylic

acids such as mevalonic acid, benzoic acid, and monocarboxylic acid forms of structural analogues, lovastatin acid and simvastatin acid, whereas di- and tricarboxylic acids or acidic amino acid were not inhibitory. Furthermore, mutual inhibitory effects were observed between pravastatin and acetic acid (69). Based on these results, a proton-coupled, monocarboxylic acid-specific transport mechanism for pravastatin is highly likely to be present in the intestine.

PHOSPHATE TRANSPORT

The carrier-mediated transport of phosphate in the small intestine was clearly shown in isolated brush-border membrane vesicles prepared from rat (70) and human intestine (71). Uptake of phosphate by the rat membrane vesicles was stimulated in the presence of an inwardly directed sodium ion gradient and was also affected by pH, with an increased activity at an acidic extravesicular pH of 6 compared with neutral pH, 7.4. There have been a few studies on intestinal basolateral membrane transport of phosphate, and a sodium-dependent transport mechanism has been suggested for rat and human intestines (71). However, the precise mechanisms involved remain unclear.

The intestinal phosphate transporters have not been cloned yet. Interestingly, no genes in mammalian intestines homologous to the renal phosphate transporter genes have been found. Accordingly, in contrast to other nutrient transporters, intestinal and renal brush-border membranes may contain structurally different phosphate transporters.

Utilization of Phosphate Transporter for Drug Absorption

The antiviral drug, foscarnet (phosphonoformic acid, structure in Fig. 2), which is water-soluble with pKa values of 0.49 and 7.27 for phosphate hydroxy groups and 3.41 for the carboxyl group, shows a very high absolute bioavailability of about 95% in rabbits, while approximately 30% absorption in mice and rats and 12 to 22% in humans have been reported (72). Such a high intestinal absorption, in spite of its hydrophilic nature, in rabbits and the highly variable availability among animal species may be ascribed to the involvement of carrier-mediated transport mechanisms. The uptake of radio-labeled foscarnet by rat intestinal brush-border membrane vesicles was sodium ion gradient-dependent, showing an overshoot phenomenon, and was activated at an acidic extravesicular pH in comparison with neutral pH (72). Furthermore, the initial rate of uptake was inhibited by unlabeled foscarnet, phosphate, and arsenate. Such a carrier-mediated transport was also observed in studies of rat intestine mounted in an Ussing-type chamber (73). All of these observations indicate that foscarnet may be absorbed in the small intestine via a carrier-mediated mechanism which is common to phosphate.

Fosfomycin, (−)-(1R,2R)-(1,2-epoxypropyl)phosphonic acid (structure in Fig. 2), is a water-soluble antibiotic, which is administered orally as well as parenterally, though its bioavailability is not high. Studies with rat, rabbit and human intestinal brush-border membrane vesicles showed that this phosphate-mimetic antibiotic is taken up by the Na^+-phosphate cotransporter (74,75). An *in situ* intestinal perfusion study suggested that the carrier-mediated absorption via the phosphate transporter is more important at concentrations of less than 1 mM fosfomycin, considering from Km value for the carrier-mediated transport of 1.13 mM (76).

Considering the structures of fosfomycin, foscarnet, and its analogues (e.g., phosphonoacetic acid and phosphonopropionic acid), relatively small molecules containing a phosphate moiety may be utilized as substrates for the intestinal sodium-dependent phosphate transporter, resulting in enhanced intestinal absorption.

BILE ACID TRANSPORT

Bile Acid Transport Mechanism in Intestinal Brush-Border Membrane

Bile acids are acidic sterols synthesized from cholesterol in the liver. Following synthesis, bile acids are secreted into bile, enter the lumen of the small intestine and are reabsorbed to the extent of more than 95% in the small intestine, predominantly by an Na^+ gradient-driven transporter located at the brush-border membrane of the ileum (77). Recently, cDNA of the bile acid-Na^+ acid cotransporter (IBAT), encoding a 348-amino acid protein with seven putative transmembrane domains and three possible N-linked glycosylation sites, was cloned (78). The amino acid sequence was 36% identical and 63% similar to that of the rat liver bile acid-Na^+ transporter (LBAT).

Utilization of Bile Acid Transporters for Drug Absorption

A series of small, linear, model peptides up to a chain length of 10 amino acids were covalently coupled to the 3-position of a modified bile acid yielding peptidyl-3β-(ω-amino-alkoxy)-7α,12α-dihydroxy-5β-cholan-24-oic acid (structure in Fig. 2). These compounds and a bile acid conjugate (S3744) were able to interact with the ileal bile acid-Na^+ cotransport system, as was shown by their concentration-dependent inhibitory effects on Na^+-dependent [^{3}H]taurocholate uptake by brush-border membrane vesicles from rabbit ileum (79).

DRUG ABSORPTION VIA WATER-SOLUBLE VITAMIN TRANSPORT SYSTEMS

Drug Absorption via Nicotinic Acid Transport System

Nicotinic acid has a single carboxyl group with a pKa of 4.9. A rat intestinal brush-border membrane vesicle study revealed a sodium-independent and proton-gradient-dependent transport system for nicotinic acid (80), although previous studies using everted sacs or perfusion of rat small intestine showed passive diffusion or Na^+-dependent transport. Nicotinic acid uptake was inhibited by several acidic compounds including acetic acid, valproic acid, benzoic acid and salicylic acid. These effects were considered to be specific, because the transport of acetic acid and benzoic acid by brush-border membrane vesicles or Caco-2 cells, respectively, was also inhibited by nicotinic acid (67,68). Nicotinic acid transport in intestinal brush-border membrane vesicles was also stimulated in the presence of an outwardly-directed bicarbonate gradient at acidic pH (81). Several lines of evidence support the hypothesis that various monocarboxylic acid-like drugs, such as valproic acid, salicylic acid, and penicillins, are absorbed via H^+-nicotinic acid cotransporter

and/or HCO_3^-/nicotinic acid exchanger as well as lactic acid and/or SCFA transporters, as described in the previous section.

Transport of Folic Acid and Its Analogues

Folic acid is likely to be absorbed mainly via a pH-dependent carrier-mediated transport mechanism (82). In rat, rabbit, and human intestinal brush-border membrane vesicles, an inwardly-directed proton-gradient induced overshoot uptake of folic acid, and the uptake was saturable and inhibited by an anion exchange inhibitor, DIDS. These results were explained in terms of a folic acid-hydroxyl exchange or proton-cotransport mechanism (83). Basolateral membrane transport of folic acid was reported to be saturable, electroneutral, sodium-independent and sensitive to DIDS (84).

Methotrexate is an analogue of folic acid and its intestinal absorption mechanism is similar to that of folic acid. However, it has also been suggested that there are multiple pathways for methotrexate, one being common with folic acid and the other specific to methotrexate due to the presence of folic acid-insensitive flux as a major component of the total flux of methotrexate, though the precise mechanism of the transport has not been clarified yet (85). Although potocytosis has also been suggested in an internalization of folic acid via folic acid receptor (86), it is not clear whether the mechanism functions in intestinal epithelial cells for the absorption of methotrexate or not.

Drug Absorption via Choline Transport System

Recent studies using isolated brush-border membrane vesicles confirmed a facilitated transport of choline in rats (87). Uptake of choline by membrane vesicles was not sensitive to an inwardly directed sodium or proton gradient, membrane potential or outwardly directed proton gradient, but was saturable with a Km of 159 μM. Tetraethylammonium, acetylcholine, and N-methylnicotinamide, which are comparatively water-soluble, small organic cations, most likely share a common transporter with choline, whereas hydrophobic hexyltrimethylammonium and octyltrimethylammonium probably do not (87). Furthermore, the presence of a carboxyl group in choline analogues, including betaine, carnitine, sarcosine, and N,N'-dimethylglycine, decreases affinity for the choline transporter (88).

DRUG ABSORPTION LIMITED BY P-GLYCOPROTEIN-MEDIATED SECRETORY DRUG TRANSPORT

P-Glycoprotein (P-gp, the putative structure of human *MDR1* gene is shown in Fig. 4) is a transmembrane protein of 170 kDa associated with a phenotype of multidrug resistance (MDR) of tumor cells to certain anticancer agents through pumping the agents out of the cells, thereby reducing the intracellular accumulation of the drugs. Functionally, P-gp is characterized by a surprisingly broad substrate specificity, including anticancer drugs, calcium channel blockers, immunosuppressive agents and others, and is classified as an ATP-dependent primary active transporter belonging to the ABC (ATP binding cassette) transporter superfamily. Furthermore, P-gp has been shown to be present and to function as a transporter in plasma membrane of many normal tissues (89,90). The functional significance of P-gp as the drug efflux pump in some normal tissues was clearly demonstrated by generating a mouse strain in which the mdr1a gene encoding P-gp was disrupted. In the mouse lacking the mdr1a gene product, distribution of an anticancer drug, vinblastine, and an anthelmintic agent, ivermectin, was enhanced in many tissues especially in the brain (91), and this result supports our previous conclusion that P-gp has a function in maintaining the blood-brain barrier (92–95). Intestinal tissue distributions of vinblastine and ivermectin were also increased in the mutant mouse (91). This increase is consistent with the previous findings that P-gp is localized on the luminal membrane of intestinal epithelial cells and transports anticancer drugs such as anthracyclins (96–98) and etoposide (99), antibiotic agents such as pristinamycin (100), peptides such as cyclosporin A (101) and model peptides (102), β-blockers such as celiprolol (103) and other organic cations (104,105). These results have been obtained by measurements of the polarized transport from the basolateral to apical side across isolated intestinal tissues or cultured Caco-2 cells and the effect of classical P-gp inhibitors such as verapamil and functionally blocking-type anti-P-gp antibody on the transepithelial transport (106).

From the above data, it is clear that P-gp functions to reduce apparent intestinal epithelial permeability from lumen to blood for various lipophilic or cytotoxic drugs. Figure 9 shows a summary of the relationship between absorption clearance evaluated by the rat intestinal perfusion or loop method and lipid solubility of the drugs (107–109). Here, closed squares represent the absorption clearance obtained in the case of simultaneous intravenous administration of cyclosporin A to block the function of P-gp. The compounds examined include β-blockers (atenolol, acebutolol, celiprolol and nadolol), anticancer drugs (doxorubicin and vinblastine) and other P-gp substrates (cyclosporin A, digoxin and verapamil). The solid line represents a visually fitted correlation curve for the drugs shown by circles. Intestinal absorption of drugs involved in the multidrug-resistance phenotype tends to be increased to some extent by cyclosporin A administration, although the increased rate constants still seem to be lower than those expected from the apparent correlation (solid line) (unpublished observations in our laboratory). Thus, P-gp primarily decrease net intestinal absorption of some drugs, although other secretory mechanisms may also contribute to the reduction of apparent intestinal permeability.

CONCLUSION

This review focuses on the experimental basis for the modern approach to carrier-mediated intestinal absorption/secretion of drugs, rather than the classical approach of passive absorption by intestinal tract by increasing the lipophilicity of drugs. In recent years, transport proteins for nutrients have been established to play an important role in regulating the intestinal absorption of xenobiotics. An understanding of the functional characteristics of such transporters should provide information on how transporters contribute to the increased bioavailability of xenobiotic compounds, facilitating the design of new orally effective drugs.

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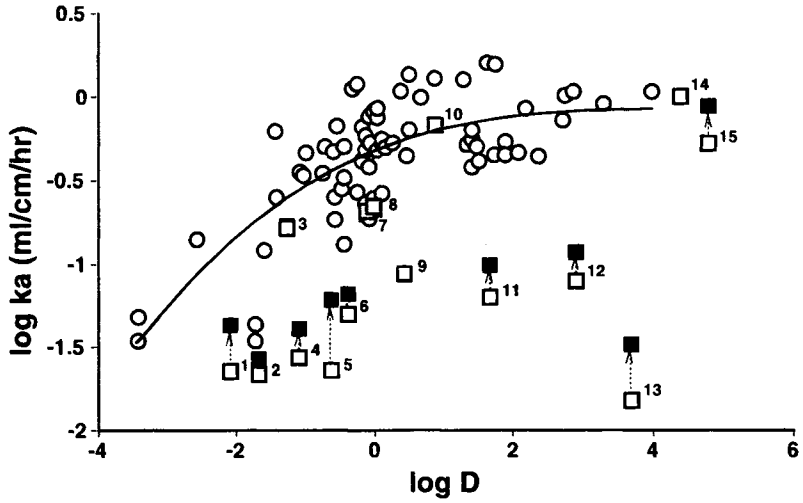

Fig. 9. Relationship between rat intestinal absorption clearance and lipid solubility. The results shown with the squares represent the relationship between intestinal absorption clearance (ka) observed from the in situ jejunum loop in the presence (■) and absence (□) of cyclosporin A in rats and octanol-buffer (pH 7.0) partition coefficients (log D). determined in this study. The results shown with circles were obtained from Refs. 106, 107, and 108. Other data points were determined by our laboratory which include 1, atenolol; 2, nadolol; 3, acetamide; 4, celiprolol; 5, acebutolol; 6, doxorubicin; 7, timolol; 8, sulfathiazole; 9, quinidine; 10, sulfamethoxazole; 11, digoxin; 12, cyclosporin A; 13, vinblastine; 14, β-estradiol; 15, verapamil.

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