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Secretory transport of *p*-aminohippuric acid across intestinal epithelial cells in Caco-2 cells and isolated intestinal tissue

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Abstract

The intestinal transport of an organic anion, *p*-aminohippuric acid (PAH), was studied in Caco-2 cell monolayers and rat intestinal tissue mounted in Ussing chambers.

In both experimental methods, PAH exhibited vectorial transport with significantly greater permeability in the secretory direction than the absorptive direction, indicating net secretion. This secretory transport required metabolic energy, but protons or hydroxyl ions were not involved as the driving force. In Caco-2 monolayers, secretory transport of [^{3}H]PAH was decreased, and the intracellular accumulation of PAH was increased with increasing concentration of unlabelled PAH at the basolateral side. Addition of probenecid and genistein at the basolateral side decreased the secretory transport of [^{3}H]PAH; the accumulation was not changed by probenecid, but was increased by genistein. In addition, the initial uptake rate of [^{3}H]PAH from the basolateral side was decreased by both PAH and probenecid, but not by genistein. Therefore, it is suggested that the transport of PAH in Caco-2 cells is regulated by several transporters: a genistein-sensitive transporter on the apical membrane and probenecid-sensitive transporters on both the basolateral and apical membranes. In rat intestinal tissues, the transport rate of PAH showed regional variation (ileum > jejunum > duodenum), suggesting that secretory transporters with high activity exist predominantly in the lower region of the small intestine.

The results suggest that PAH transport in both Caco-2 cells and rat intestinal tissues is regulated by multiple transporters on the apical and basolateral membranes, and these transporters have different characteristics.

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Introduction

There are many studies focusing on intestinal absorption mechanisms of drugs, including both passive diffusion, based on the physicochemical properties of drug molecules, and selective transport systems mediated by specific transporters. The variable bioavailability of drugs after oral administration might be understandable, at least in part, by determining if they are substrates for absorptive transporters or not (Tsuji & Tamai 1996). Several transporters, which are involved in absorption, have been cloned and their characterization has contributed to our understanding of the mechanisms of drug absorption (Tsuji & Tamai 1996).

The occurrence of efflux/secretion into the intestinal lumen has been demonstrated. Several efflux transporters with broad substrate specificity were reported to

be expressed in the intestine, including P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and the polyspecific cation transporter, OCT1 (Grundemann et al 1994; Barrand et al 1997). P-gp functions as an efflux pump on the apical membrane of small intestinal epithelial cells as well as other cells, including cancer cells (Gatmaitan & Arias 1993). Recently, it has been reported that some drugs administered intravenously are secreted into the intestinal lumen in considerable amounts (Mayer et al 1996, 1997). We demonstrated that the intestinal absorption of a serotonin (5-HT) antagonist, azasetron, is regulated by absorptive and secretory transporters (Tamai et al 1997). It has also been reported that the intestinal absorption of some compounds is limited partly because they are preferentially transported in the secretory direction by P-gp (Saitoh & Aungst 1995; Terao et al 1996; Wacher et al 1998). In addition to the efflux transport of cationic or neutral compounds by P-gp, anionic compounds are also hypothesized to be secreted into the intestinal lumen. It was demonstrated in Ussing-chamber studies that cefazolin, phenol red, and calcein are preferentially transported in the secretory direction (Saitoh et al 1996; Fujita et al 1997). Moreover, MRP2/cMOAT (canalicular multispecific organic anion transporter), is expressed in the small intestine (Paulusma et al 1996; Kool et al 1997), and is suggested to exist on the apical membrane of intestinal epithelial cells (Hirohashi et al 2000). Although molecular and functional studies of MRP2 in the liver revealed that the transporter is responsible for biliary excretion of many organic anionic compounds, including conjugated metabolites, across the canalicular membrane (Oude et al 1995; Yamazaki et al 1996), only little information on its function in the small intestine is available (Walle et al 1999).

Both P-gp and MRP2 exhibit broad substrate specificities, and inhibitors of the transporters are diverse. MRP2 tends to have high affinity for anionic compounds (Oude et al 1995; Yamazaki et al 1996), whereas P-gp transports neutral or cationic drugs (Kusuhara et al 1998). Although many investigators have demonstrated that various organic cationic compounds are excreted almost exclusively via P-gp, the number of studies on intestinal secretion of other organic anions is still limited, and the precise mechanisms remain unclear. Therefore, it is important to clarify the transport mechanisms operating in the intestine.

In addition to the transporters identified in the intestine, many transporters contributing to efflux/secretory transport exist in other tissues. *p*-Aminohippuric acid (PAH) is an endogenous end product and is exclusively eliminated through the kidneys. The renal epithelial membrane transport of PAH involves exchange transport with dicarboxylic acid at the basolateral membrane via the OAT transporter (Sekine et al 1997). Since PAH is a typical substrate for organic anion transporters, we used it as a model substrate to clarify the transport characteristics of organic anions in intestinal epithelial cells.

Materials and Methods

Materials

[^{3}H]PAH (47.4 GBq mmol^{-1}) and [^{14}C]mannitol (1.9 GBq mmol^{-1}) were purchased from New England Nuclear (Boston, MA). All other chemicals used were obtained commercially and were of reagent grade.

Transport experiments with Caco-2 cells

Caco-2 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1% non-essential amino acids, 2 mM L-glutamine, 100 U mL^{-1} penicillin G and 100 μg mL^{-1} streptomycin, as described previously (Tsuji et al 1994). Caco-2 cells were grown on Transwell microporous polycarbonate membrane (Costar, Bedford, MA) for 21–23 days and the transepithelial electrical resistance of the monolayer was 250–350 $\Omega \cdot$ cm^2 when used for transport experiments. The transport study was performed as described previously (Tsuji et al 1994). The confluent cells were washed with Hanks-balanced salt solution (HBSS) (mM: 0.952 $CaCl_2$, 5.36 KCl, 0.441 KH_2PO_4, 0.812 $MgSO_4$, 136.7 NaCl, 0.385 Na_2HPO_4, 25 D-glucose and 10 HEPES, pH 7.4 or 6.0, osmolarity 315 mOsm kg^{-1}) at 37°C (except for the temperature-dependence experiment), and 0.5 and 1.5 mL HBSS were added on the apical and basolateral sides of a cell insert, respectively. To measure apical-to-basolateral (a-to-b) or basolateral-to-apical (b-to-a) flux, a test compound was included in the apical or basolateral side, respectively. At the designated time, 0.5 mL of the basolateral or 0.2 mL of the apical side solution was withdrawn and replaced with an equal volume of HBSS. At the end of the transport experiments, the membrane was washed three times with ice-cold HBSS, cut and solubilized in 500 μL 5 M NaOH for 2 h. After neutralization with 500 μL 5 M HCl, radioactivity associated with the cells was measured.

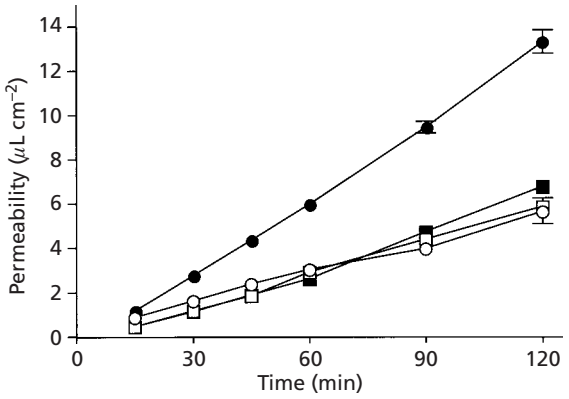

Figure 1 Permeation of [^{3}H]PAH and [^{14}C]mannitol across Caco-2 cell monolayers. Transport of [^{3}H]PAH (○) and [^{14}C]mannitol (□) in the apical-to-basolateral direction and transport of [^{3}H]PAH (●) and [^{14}C]mannitol (■) in the basolateral-to-apical direction. The experimental solution was adjusted to pH 7.4 and the temperature was maintained at 37°C. The concentrations of [^{3}H]PAH and [^{14}C]mannitol were 0.62 μM and 3.88 μM, respectively. Each point represents the mean ± s.e. of three experiments.

Transport experiments by the Ussing-type chamber method

Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). Animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals, Takara-machi Campus of Kanazawa University. Rat intestinal tissue sheets were prepared as described previously (Tamai et al 1997). The tissue preparation, consisting of the mucosa and most of the muscularis mucosa, was made by removing the submucosa and tunica muscularis with fine forceps. The tissue sheets were mounted vertically in an Ussing-type chamber that provided an exposed area of 0.5 cm^2. The volume of bathing solution on each side was 5 mL, and the solution temperature was maintained at 37°C in a water-jacketed reservoir (except for the temperature-dependence experiment). The test solution was composed of (mM): 128 NaCl, 5.1 KCl, 1.4 $CaCl_2$, 1.3 $MgSO_4$, 21 $NaHCO_3$, 1.3 KH_2PO_4, 10 NaH_2PO_4 and 5 glucose, pH 7.4 or 6.0, and the solution was gassed with 95% O_2/5% CO_2 before and during the transport experiment.

Analytical methods

To assay radioactivity, all samples were transferred into counting vials, mixed with scintillation fluid (Cleasol I; Nacalai Tesque, Kyoto, Japan) and quantified in a liquid scintillation counter (Aloka, Tokyo, Japan). Non-radioactive PAH was used in the Ussing chamber method and the amount transported was measured by HPLC. The HPLC system consisted of a constant-flow pump (880-PU; Japan Spectroscopic Co., Tokyo, Japan), a UV detector (UV 970; Japan Spectroscopic Co.), integrator (Chromatopac CR3A; Shimadzu Co., Kyoto, Japan) and an automatic sample injector (AS-L350; Japan Spectroscopic Co.). The UV detector was set at 274 nm, and a Cosmosil 5C_{18}-MS (4.6 x 150 mm; Nacalai Tesque, Kyoto, Japan) was used at room temperature. The mobile phase consisted of 10 mM KNO_3–acetic acid-acetonitrile (100:1:2, v/v), and the flow rate was 1.0 mL min^{-1}. The calibration curve was linear over the concentration range 0.5–500 μM. The within- and between-day coefficients of variation for the assay were less than 8%.

Data analysis

Permeation or accumulation (μL cm^{-2}) was estimated by dividing the amount transported or accumulated by the initial concentration of test compound on the donor side. The permeability coefficient or accumulation rate (μL cm^{-2} min^{-1}) was obtained from the slope of the linear portion of the plots of permeation or accumulation against time. All data are expressed as means ± s.e. and statistical analysis was performed using the Student's t-test. A difference between means was considered to be significant at $P < 0.05$.

Results

PAH transport in Caco-2 cells

To determine if the transport of [^{3}H]PAH across Caco-2 cells was unidirectional, transepithelial fluxes were measured by adding [^{3}H]PAH (0.62 μM) to either the apical or basolateral side of monolayers of Caco-2 cells, and monitoring the appearance of radioactivity on the opposite side (Figure 1). The b-to-a permeability coefficient of [^{3}H]PAH was significantly higher than the a-to-b permeability coefficient. The a-to-b permeability coefficient of [^{3}H]PAH was comparable with that of [^{14}C] mannitol in either direction, which is a measure of paracellular permeability, suggesting that absorptive transport of [^{3}H]PAH is only via the paracellular route. Therefore, the [^{3}H]PAH transport in the secretory direction could be attributed to transcellular permeation. In addition, accumulation of [^{3}H]PAH from the basolateral side (1.63 ± 0.13 μL cm^{-2}) was significantly

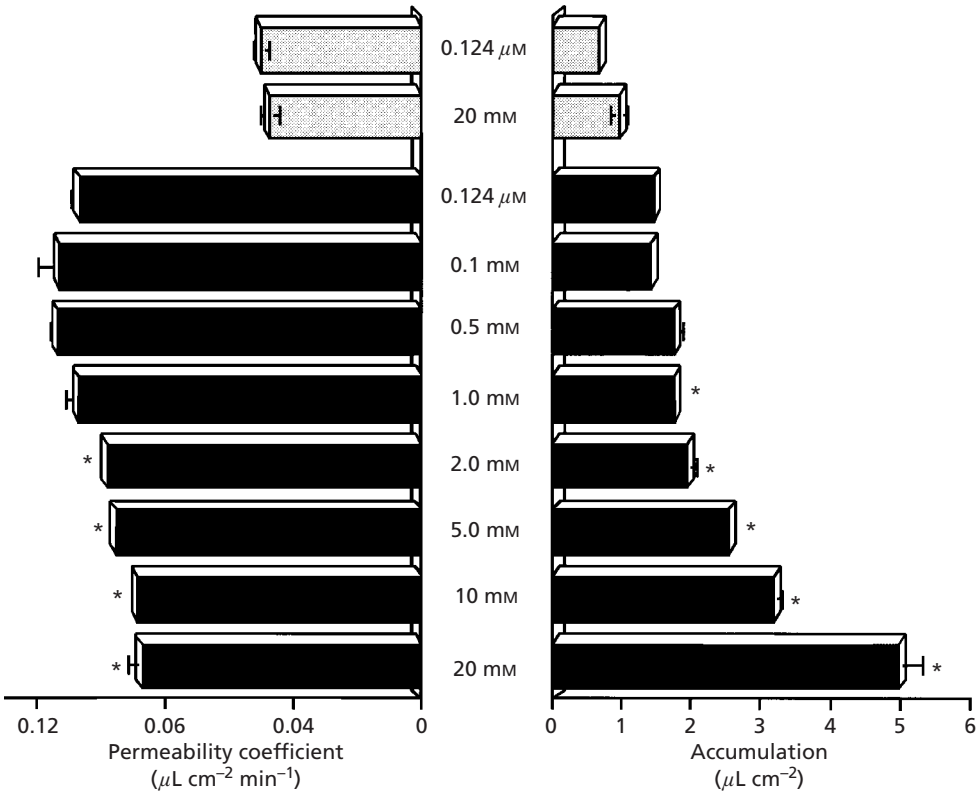

Figure 2 Effect of unlabelled PAH on [^{3}H]PAH permeation across, and accumulation in, Caco-2 cell monolayers. Dotted and closed columns represent the apical-to-basolateral and basolateral-to-apical (b-to-a) directions, respectively. Transport of [^{3}H]PAH (0.124 μM) across Caco-2 cells was evaluated from the time course of amount transported to the receiver side. Unlabelled PAH was added only in the donor bathing solution. Accumulation was measured after the experimental time period of 120 min. The experimental solution was adjusted to pH 7.4 and maintained at 37°C. Each column represents the mean $\pm$ s.e. of three experiments. *$P < 0.05$ significantly different compared with b-to-a (0.124 μM).

Table 1 Effects of pH and temperature on [^{3}H]PAH permeation across Caco-2 cell monolayers.

Parameter		Substrate	Permeability coefficient (μL cm^{-2} min^{-1})	
			a-to-b	b-to-a
Extracellular pH (apical/basolateral)	7.4/7.4	[^{3}H]PAH	0.043 $\pm$ 0.003	0.151 $\pm$ 0.009
	6.0/7.4		0.037 $\pm$ 0.002	0.128 $\pm$ 0.006
	7.4/6.0		0.051 $\pm$ 0.009	0.161 $\pm$ 0.013
Temperature	37°C	[^{3}H]PAH	0.049 $\pm$ 0.002	0.124 $\pm$ 0.001
	4°C		0.015 $\pm$ 0.001*	0.019 $\pm$ 0.001*
	37°C	[^{14}C]Mannitol	0.039 $\pm$ 0.002	0.048 $\pm$ 0.002
	4°C		0.016 $\pm$ 0.001*	0.015 $\pm$ 0.003*

a-to-b and b-to-a refer to apical-to-basolateral and basolateral-to-apical directions, respectively. In pH-dependence experiments, permeation of [^{3}H]PAH (0.124 μM) was measured at 37°C for 120 min. In temperature-dependence experiments, the experimental solution was adjusted to pH 7.4 and permeation of [^{3}H]PAH (0.62 μM) and [^{14}C]mannitol (3.88 μM) were measured for 120 min. Data represent the mean $\pm$ s.e. of three experiments. *$P < 0.05$, significantly different compared with the permeability at 37°C. No significant difference was observed between a-to-b and b-to-a permeability of either [^{3}H]PAH or [^{14}C]mannitol at 4°C.

Table 2 Effect of various compounds on [^{3}H]PAH secretory permeation across, and accumulation in, Caco-2 cell monolayers.

Inhibitor	Concentration	Permeability coefficient (% of control)	Accumulation (% of control)
Control	–	100.0	100.0
Ciclosporin	1 μM	114.0 ± 10.3	104.5 ± 2.6
Genistein	200 μM	61.0 ± 2.7*	197.1 ± 2.5*
Probenecid	5 mM	34.2 ± 7.7*	101.7 ± 16.0
Leukotriene C_4	100 nM	104.3 ± 2.7	88.0 ± 9.2
Glutarate	10 mM	100.5 ± 3.2	104.7 ± 13.4
Benzylpenicillin	10 mM	102.9 ± 3.2	113.7 ± 4.5
Triiodothyronine	40 μM	107.9 ± 4.9	106.1 ± 5.1

The experimental solution was double-labelled with [^{3}H]PAH (0.62 μM) and [^{14}C]mannitol (3.88 μM), and the pH was adjusted to 7.4. Permeation and accumulation of [^{3}H]PAH (0.62 μM) were measured at 37°C for 120 min. Transport was evaluated in terms of the permeability coefficient of [^{3}H]PAH minus that of [^{14}C]mannitol and calculated as a percentage of the control. Each value represents the mean ± s.e. of three experiments. *$P < 0.05$, significantly different compared with the control.

Table 3 Effect of unlabelled PAH and various compounds on uptake of [^{3}H]PAH from the basolateral side in Caco-2 cell monolayers.

Inhibitor	Concentration	Uptake ratio (% of control)
Control	–	100.0
PAH	0.5 mM	72.6 ± 23.6
PAH	30 mM	48.5 ± 13.4*
Probenecid	5 mM	55.0 ± 16.9*
Genistein	200 μM	84.6 ± 22.1

Uptake of [^{3}H]PAH (0.124 μM) was measured at 1, 2 and 5 min (n = 3). The experimental solution was adjusted to pH 7.4 and maintained at 37°C. The slope of the nine points of accumulated amount vs time curve, obtained by linear regression analysis, was defined as the uptake rate. *$P < 0.05$, significantly different compared with the control.

higher than that from the apical side (0.67 ± 0.04 μL cm^{-2}) at 120 min.

The effect of increasing concentrations of unlabelled PAH on the transport and accumulation of [^{3}H]PAH (0.124 μM) in Caco-2 cells was studied (Figure 2). A high concentration of unlabelled PAH (2 mM) did not affect either the a-to-b permeability coefficient or accumulation from the apical side up to 120 min. Since it is considered that the absorptive transport is not mediated by any transporter as mentioned above, no saturation occurred. The b-to-a permeability coefficient of [^{3}H]PAH was saturable over 2 mM. Moreover, [^{3}H]PAH accumulation from the basolateral side up to 120 min increased with increasing PAH concentration.

The effect of protons on [^{3}H]PAH permeability coefficient and accumulation up to 120 min was examined by changing the extracellular pH of HBSS (Table 1). The effect of pH was negligible, suggesting that protons and hydroxyl ions are not involved as the driving force of PAH transport in Caco-2 cells. Therefore, the following experiments were performed at pH 7.4.

To determine if the [^{3}H]PAH transport requires metabolic energy, its temperature-dependence was studied (Table 1). Lowering the temperature to 4°C significantly decreased the [^{3}H]PAH permeability coefficients in both directions to values comparable with those of [^{14}C]-mannitol at 4°C. In particular, strong temperature-dependence was observed in b-to-a transport.

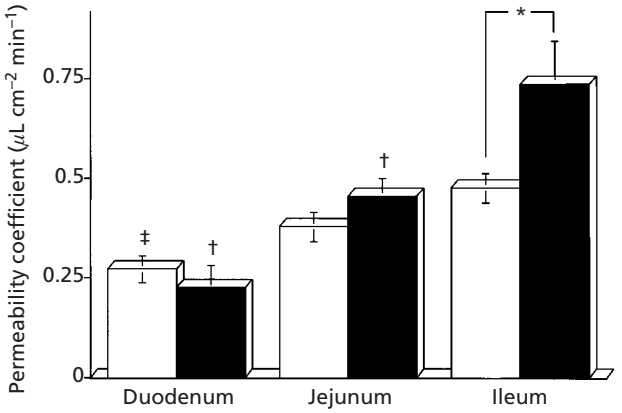

Figure 3 PAH transport across rat intestinal tissue from various regions. The transport of PAH (0.1 mM) across the intestinal tissue was evaluated from the time course of the transport by the Ussing chamber method. The tissue was isolated from duodenum, jejunum or ileum. The experimental solution was adjusted to pH 7.4 and the temperature was maintained at 37°C. Dotted and closed columns represent the mucosal-to-serosal (m-to-s) and serosal-to-mucosal (s-to-m) directions, respectively. Each column represents the mean ± s.e. of three to six experiments. *$P < 0.05$, significant difference between m-to-s and s-to-m transport in the ileum. †$P < 0.05$ and ‡$P < 0.05$, significant difference compared with s-to-m and m-to-s transport in the ileum, respectively.

To clarify the characteristics of transporters responsible for the secretory transport of PAH, the inhibitory effects of several compounds on the transport and accumulation of [^{3}H]PAH in Caco-2 cells from the basolateral side were studied. As shown in Table 2,

Table 4 Concentration-dependence of PAH transport in rat ileal tissue.

Concentration (mM)	Permeability coefficient (μL cm^{-2} min^{-1})			
	m-to-s	n	s-to-m	n
0.1	0.474 ± 0.036	6	0.734 ± 0.110	6
1	0.555 ± 0.048	4	0.833 ± 0.089	4
5	0.481 ± 0.040	4	0.662 ± 0.108	4
10	0.315 ± 0.014*	5	0.659 ± 0.020†	4

m-to-s and s-to-m refer to the mucosal-to-serosal and serosal-to-mucosal directions, respectively. The ileal transport was evaluated at concentrations of 0.1–10 mM by the Ussing chamber method. The experimental solution was adjusted to pH 7.4 after addition of the indicated concentration of PAH and the temperature was maintained at 37°C. Each value represents the mean ± s.e. of the indicated number of experiments (n). * $P < 0.05$ and † $P < 0.05$ significantly different compared with m-to-s (0.1 mM) and s-to-m (1 mM), respectively.

genistein significantly decreased the b-to-a permeability coefficient of [^{3}H]PAH and significantly increased the accumulation. Probenecid significantly decreased the b-to-a permeability coefficient, but did not influence the accumulation up to 120 min. To further characterize the mechanisms of PAH transport across the basolateral membrane, the uptake rate of [^{3}H]PAH from the basolateral side over 5 min was measured (Table 3). The uptake rate of [^{3}H]PAH over 5 min from the basolateral side was dose-dependently decreased by PAH. Genistein had little effect on the rate, but probenecid decreased it considerably.

PAH transport in rat small intestinal tissue

We studied PAH permeation in rat intestinal tissue mounted in Ussing chambers to determine if the phenomena seen in Caco-2 cells are also observed under these conditions. Permeability coefficients of PAH in both directions showed regional dependence with an increase in the order of duodenum < jejunum < ileum (Figure 3). Serosal-to-mucosal (s-to-m) permeability coefficients of PAH were greater than mucosal-to-serosal (m-to-s) permeability coefficients and this secretory transport was statistically significant in the lower region (ileum). This suggests that secretory transport activity is high in the lower region of the small intestine. Therefore, ileum was used in the following experiments. The relationship between permeability coefficients and the concentration of PAH was evaluated using ileal tissue (Table 4). The permeability coefficients in both directions tended to increase at low concentrations (approx. 1 mM) and to decrease at higher concentrations (approx. 5 mM). The extracellular pH was changed to evaluate the effects of protons or hydroxyl ions on PAH transport. As observed in Caco-2 cells, extracellular pH did not affect the permeability coefficients of PAH in rat ileum (Table 5), indicating that protons and hydroxyl ions are not important for PAH transport in rat intestinal tissue. Therefore, the following experiments were performed at pH 7.4.

To clarify if the efflux transport was energy-dependent, the transport of PAH was measured at 4°C. PAH permeability coefficients at 4°C in both directions were decreased to the same level and the unidirectionality disappeared (Table 5).

Table 5 Effects of pH and temperature on PAH transport in rat ileal tissue.

Parameter		Permeability coefficient (μL cm^{-2} min^{-1})			
		m-to-s	n	s-to-m	n
Extracellular pH (mucosal/serosal)	7.4/7.4	0.474 ± 0.036	6	0.734 ± 0.110	6
	6.0/7.4	0.432 ± 0.044	4	0.669 ± 0.102	3
	7.4/6.0	0.427 ± 0.058	4	0.618 ± 0.009	3
	6.0/6.0	0.467 ± 0.034	5	0.731 ± 0.099	4
Temperature	37°C	0.474 ± 0.036	6	0.734 ± 0.110	6
	4°C	0.347 ± 0.049*	6	0.275 ± 0.016*	4

m-to-s and s-to-m refer to the mucosal-to-serosal and serosal-to-mucosal directions, respectively. In pH-dependence experiments, the experimental solution was adjusted to different pH values as indicated, and permeation of PAH (0.1 mM) was measured at 37°C for 130 min. In temperature-dependence experiments, the experimental solution was adjusted to pH 7.4 at 4 or 37°C and permeation of PAH (0.1 mM) was measured for 130 min. Each value represents the mean ± s.e. of the indicated number of experiments (n). In pH-dependency experiments, no significant difference was observed within m-to-s or s-to-m groups. * $P < 0.05$ significantly different compared with the permeability at 37°C.

Discussion

Drug absorption from the intestine or oral bioavailability is determined by various mechanisms. The high oral bioavailability of drugs has been explained, in part, by the existence of absorptive transporters in addition to passive diffusion mechanisms (Tsuji & Tamai 1996).

Recent studies support the concept that certain drugs are preferentially secreted into the intestinal lumen from the blood, and that this secretory transport limits the oral bioavailability of certain drugs (Mayer et al 1996, 1997). Some drugs, such as ciclosporin and verapamil, have been reported to be secreted via P-gp in the intestine (Saitoh & Aungst 1995; Terao et al 1996). Recently, it has been reported that MRP2 is involved in the secretion of organic anions in the small intestine (Gotoh et al 2000). Nevertheless, there is little information on the secretion of anionic compounds in the intestine. Therefore, it is of interest to investigate the transport systems that contribute to the secretory transport of anionic compounds in the intestine. In this study, we used PAH as a model anionic compound, because the transport of [^{3}H]PAH across monolayers of Caco-2 cells was secretory-directed and the absorptive transport was comparable with the transport of [^{14}C]mannitol in both directions (a measure of paracellular transport) (Figure 1). Therefore, it is considered that secretory transporters, rather than absorptive transporters, are important in determining the intestinal transport of PAH.

The properties of PAH transport across Caco-2 cells were very similar to those of PAH transport across the rat intestinal tissue. PAH transport was secretory-directed (Figures 1 and 3), pH-independent and temperature-dependent (Tables 1 and 5) in both Caco-2 cells and rat intestinal tissue, suggesting that secretory transporters are involved in PAH transepithelial permeation. The values of activation energy evaluated from the temperature-dependence experiments of PAH transports in Caco-2 cells and rat tissue were 9.7 and 5.1 kcal mol^{-1} for b-to-a and s-to-m directions, respectively, and were greater than that explained by simple diffusion, indicating carrier-mediated efflux transport mechanisms (Hidalgo & Borchardt 1990; Makhey et al 1998). Therefore, the transporters are likely to require metabolic energy, but protons or hydroxyl ions are unlikely to be the driving force for the PAH transport. Since similar phenomena were observed in both Caco-2 cells and rat intestinal tissue, it is likely that humans and rats share the same or very similar transporters for PAH transepithelial transport. The absorptive permeability coefficient varied with concentration in the rat intestinal tissue (Table 4), whereas no significant alteration was observed in Caco-2 cells (Figure 2). Therefore, rat intestine might have a transporter for absorptive transport of PAH which is absent, or nearly so, in Caco-2 cells. Moreover, the transport rates of PAH across intestinal tissues mounted in Ussing chambers showed regional difference, increasing in the order of duodenum $<$ jejunum $<$ ileum (Figure 3). This result suggests that secretory transporters with high activity exist predominantly in the lower region of the small intestine.

To focus on the secretory mechanisms of PAH transport, an inhibition study was carried out using Caco-2 cells. Secretory transport systems in the intestinal epithelial cells may have similar characteristics to those identified in other organs. Therefore, to clarify the possible contribution of known transporters to PAH transport in Caco-2 cells, inhibition studies were carried out using representative substrates and inhibitors of identified transporters (Table 2). It is well known that PAH is actively secreted into urine from the kidney, and this transepithelial transport in the kidney is mediated by at least two transporters. One is OAT1, which is considered to exist on the basolateral membrane. It countertransports PAH and glutarate (Sekine et al 1997). When glutarate or benzylpenicillin, both of which are inhibitors of PAH transport by OAT1, was added on the donor (basolateral) side, PAH transport and accumulation were not affected (Table 2). Moreover, even when the Caco-2 cells were pre-loaded with glutarate to increase its concentration inside the cells, PAH transport was not enhanced, and the accumulation of PAH was not decreased (data not shown). These results suggest that PAH transport is not mediated by members of the OAT family in Caco-2 cells. The organic anion transporting polypeptide (oatp) family has been identified in the liver and other organs and is known to transport anionic and neutral compounds. Triiodothyronine, which is a good substrate for oatp2/3 (Abe et al 1998), had no influence on PAH transport and accumulation. Therefore, there is little possibility that these transporters or other similar transporters participate in PAH transepithelial transport in Caco-2 cells.

P-gp and MRP2 are transporters that have already been confirmed to exist in the small intestine. P-gp favours neutral and cationic compounds, and ciclosporin is a representative substrate. Ciclosporin did not affect the transport of PAH. For MRP2, genistein (Versantvoort et al 1993; Hóllo et al 1996) and probenecid (Hóllo et al 1996; Gollapudi et al 1997) are good inhibitors, and leukotriene C_4 (Leier et al 1994; Loe et al 1996) is a good substrate. In the present study, these three compounds affected the PAH transport and

accumulation in different ways. Genistein decreased b-to-a transport of PAH and increased PAH accumulation from the basolateral side, suggesting that genistein inhibited transport on the apical membrane but not that on the basolateral membrane. Recently, transporters for PAH other than OAT were found, including Npt1 and MRP2 (Leier et al 2000). Npt is presumed to be expressed in the kidneys and liver, whereas MRP2 is present in intestinal epithelial cells in addition to liver. Therefore, MRP2 may be involved in the PAH secretion in this study. Probenecid decreased PAH transport but did not alter PAH accumulation, indicating that probenecid inhibited the transport across the basolateral membrane. However, this does not mean the result can rule out its effect on the transport across the apical membrane. To further investigate the mechanisms of PAH transport through the basolateral membrane, the initial uptake of [^{3}H]PAH from the basolateral membrane was measured (Table 3). The uptake rate decreased with increasing PAH concentration. Of the two compounds which changed the permeability coefficient and/or accumulation of [^{3}H]PAH, only probenecid decreased the initial uptake rate of [^{3}H]PAH during the first 5 min, suggesting that the transporter which is responsible for PAH transport through the basolateral membrane (i.e. transport from the basolateral side into the cell) is probenecid-sensitive. Probenecid has been shown to inhibit carrier-mediated transport of various organic anions (Takasawa et al 1997; Steffgen et al 1999). Based on the different inhibitory effects of genistein and probenecid, the transporters on the apical membrane and basolateral membrane seem to be distinct. Although the transporter sensitive to probenecid on the basolateral membrane is unknown, the transporter sensitive to genistein on the apical membrane may involve MRP2 since genistein is an MRP2 inhibitor and MRP2 is present on the intestinal apical membrane. Leukotriene C_4 is a very good substrate for MRP2, but did not affect PAH transport or accumulation in this study. The reason for this is unclear.

Summary

PAH, an organic anionic compound, is preferentially transported in the secretory direction in the intestine and this process is mediated by multiple transporters with different characteristics on the apical and basolateral membranes. The PAH transport from inside the cell to the apical side would be mediated by an MRP2-like transporter on the apical membrane. The results suggest that some anionic compounds may also be transported in the secretory direction via multiple transporters, resulting in poor bioavailability. In addition to P-gp and MRP2, which contribute greatly to the intestinal excretion of various drugs, several other transporters are possibly involved in intestinal excretion. The secretory transport activity is different at each region of the small intestine in rats. The barrier effect by these efflux transporters may be one of the critical factors limiting to the bioavailability of certain drugs.

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