Transepithelial Transport of Diphenhydramine Across Monolayers of the Human Intestinal Epithelial Cell Line Caco-2

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Received October 13, 1999; accepted February 11, 2000

Purpose. The transepithelial transport characteristics of the antihistamine, diphenhydramine, were studied in human intestinal Caco-2 cell monolayers to elucidate the mechanisms of its intestinal absorption.

Methods. The transepithelial transport and the cellular accumulation of diphenhydramine were measured using Caco-2 cell monolayers grown in Transwell chambers.

Results. The transepithelial transport of diphenhydramine from the apical to basolateral side was saturable, and the flux and cellular accumulation of diphenhydramine were dependent on the apical extracellular pH (pH 7.4 > 6.5 > 5.5). Transport and accumulation of diphenhydramine from the apical side were inhibited by another antihistamine, chlorpheniramine, while typical substrates for the renal organic cation transport system such as tetraethylammonium, cimetidine and guanidine had no effect. The transepithelial transport and cellular accumulation of diphenhydramine from the basolateral side were also pH-dependent and inhibited by chlorpheniramine. In addition, intracellular diphenhydramine preloaded was preferentially effluxed to the apical side, suggesting the involvement of the secretory pathway in diphenhydramine transport. Furthermore, diphenhydramine uptake from both the apical and basolateral sides was stimulated by preloading monolayers with chlorpheniramine (*trans*-stimulation effect).

Conclusions. Transepithelial transport of diphenhydramine across Caco-2 cells is mediated by pH-dependent, specific transport systems that exist in both the apical and basolateral membranes.

KEY WORDS: Caco-2 cells; diphenhydramine; transepithelial transport; intestinal absorption; intestinal secretion; organic cation transport.

INTRODUCTION

The mechanism of intestinal absorption of lipophilic organic cations has been explained as passive diffusion of non-ionized compounds according to the pH-partition theory unless specific transport systems are involved. Several studies have indicated that specific transport systems might facilitate the intestinal absorption of some organic cations (1–3). In these studies, saturable transport of organic cations was demonstrated. On the other hand, it was suggested that the intestinal epithelium functions as an absorptive barrier because intravenously administered drugs are excreted into the gastrointestinal tract across the intestinal membranes (4–6). Active secretion of organic cations in the intestine was first demonstrated in isolated guinea pig intestinal mucosa (7,8). Miyamoto *et al.* (9) demonstrated the presence of a guanidine/H^+ antiporter in rabbit intestinal brush-border membrane vesicles. It has been shown that P-glycoprotein localized at the intestinal brush-border membrane is involved in the active secretion of organic cations (10). However, the mechanisms of organic cation transport in the intestine are not well understood in contrast to those in kidney and liver (11,12). In addition, previous studies concerning organic cation transport in the intestine have focused mainly on the mechanisms of transport across the brush-border (apical) membrane. However, the mechanisms of organic cation transport across the intestinal basolateral membrane are poorly understood.

The human intestinal epithelial cell line Caco-2 has been used to elucidate intestinal transport mechanisms of various drugs (13). This cell line forms confluent monolayers of well-differentiated enterocyte-like cells with the functional properties of transporting epithelia (14) and has been used to study the transport of drugs. Moreover, the development of cell culture techniques using permeable supports has provided advantages in studying transepithelial transport of solutes as well as uptake characteristics across the basolateral membrane. Using Caco-2 cells grown on permeable supports, we previously characterized mechanisms of absorption of oral cephalosporins and a dipeptide-like anticancer agent, bestatin (15–17).

Recently, we demonstrated that diphenhydramine, an antihistamine, was accumulated by the pH-dependent transport system in Caco-2 cells (18). Diphenhydramine shows a relatively high absorption rate and its plasma concentration increases rapidly after oral administration (19), although the fraction of the non-ionized form is considered to be very low in the gastrointestinal tract. In the present study, we investigated the transepithelial transport and intracellular accumulation characteristics of diphenhydramine by Caco-2 cell monolayers cultured on permeable supports to clarify the mechanisms of absorption of this organic cation. Our findings suggest that pH-dependent transport systems localized in both the apical and basolateral membranes are responsible for the transepithelial flux of diphenhydramine.

MATERIALS AND METHODS

Materials

Diphenhydramine hydrochloride was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). (±)-Chlorpheniramine maleate, cimetidine, guanidine hydrochloride and tetraethylammonium bromide were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). D-[^{3}H]-mannitol (728.9 GBq/mmol) was purchased from Du Pont-New England Nuclear Research Products (Boston, MA). All other chemicals were of the highest purity available.

Cell Culture

Caco-2 cells at passage 18 obtained from the American Type Culture Collection (ATCC HTB37; Rockville, MD) were maintained by serial passage in plastic culture dishes (Falcon; Becton Dickinson & Co., Lincoln Park, NJ) as described previously (15,17). The complete medium consisted of Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Whittaker Bioproducts

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0724-8741/00/0500-0539$18.00/0

Inc., Walkersville, MD) and 1% nonessential amino acids (Gibco) without antibiotics. The cells were grown in an atmosphere of 5% CO_2–95% air at 37°C and subcultured every week using 0.02% EDTA and 0.05% trypsin.

For transport studies, Caco-2 cells were seeded on polycarbonate membrane filters (3 μm pores, 4.71 cm^2 growth area) inside Transwell cell culture chambers (Costar, Cambridge, MA) at a cell density of 3×10^5 cells/filter (6.4×10^4 cells/cm^2). Transwell culture chambers were placed in the 35 mm wells of tissue culture plates with 2.6 ml of the outside medium (basolateral side) and 1.5 ml of the inside medium (apical side). The cell monolayers were fed fresh complete medium every 2 or 3 days and were used on the 13th or 14th day for transport experiments.

To evaluate the integrity of the monolayers, we measured the transepithelial electrical resistance using Millicell-ERS (Millipore Co., Bedford, MA) in the presence of diphenhydramine (0.1–20 mM) on the apical side. The transepithelial resistance after subtracting the resistance obtained across cell-free filters was $400.0 \pm 23.2\ \Omega \cdot cm^2$ (mean ± S.E. of 15 monolayers). In addition, diphenhydramine had no effect on the apical-to-basolateral transport of D-[^{3}H]-mannitol, a marker of the paracellular transport pathway (data not shown).

Measurement of Transepithelial Transport and Cellular Accumulation

The transepithelial transport and the cellular accumulation of diphenhydramine were measured using monolayer cultures grown in Transwell chambers. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, 5 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 5.5, 6.0) or *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (pH 6.5, 7.4). In general experiments, after removal of the culture medium from both sides of the cell monolayers, the monolayers were preincubated for 10 min at 37°C with 2 ml of incubation medium on both sides. At the end of preincubation, the medium was immediately removed and then the incubation medium containing drugs was added to either the apical or the basolateral side with 2 ml of incubation medium (without drug) added to the opposite side. The incubation proceeded for specified periods of time at 37°C. To measure transepithelial transport, the incubation medium on the opposite side was collected. The collected samples were diluted 4-fold with 0.01 N HCl/methanol (1:1) and analyzed by HPLC as described below.

To measure intracellular accumulation of diphenhydramine, the medium was aspirated at the end of the incubation period and the monolayers were rapidly washed twice on both sides with 2 ml of ice-cold incubation medium (pH 7.4). The filters with cell monolayers were detached from the chambers and immersed in 0.5 ml of extraction solution (0.01 N HCl/methanol, 1:1) for 1 h at room temperature. The extraction solution was centrifuged at 13,000 rpm (Model 3533; Abbott Laboratories, Abbott park, IL) for 15 min. The supernatant was filtered through a Millipore filter (SJGVL, 0.22 μm) and analyzed by HPLC as described below.

Analytical Methods

Diphenhydramine was assayed by HPLC as previously described (18). The protein content of the cell monolayers solubilized in 1 ml of 1 N NaOH was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA) with bovine γ-globulin as the standard.

Statistical Analysis

Data were analyzed statistically by non-paired *t* test or one-way analysis of variance followed by Scheffé's test when multiple comparisons were needed. Probability values of less than 5% were considered significant.

RESULTS

Apical-to-Basolateral Transepithelial Transport and Cellular Accumulation of Diphenhydramine by Caco-2 Cell Monolayers

To characterize diphenhydramine absorption in the small intestine, transepithelial transport and cellular accumulation of diphenhydramine were examined using Caco-2 cell monolayers grown on Transwell chambers. Figure 1A shows the time-course of the apical-to-basolateral transport of diphenhydramine at various apical extracellular pHs. Both transepithelial transport (Fig. 1A) and cellular accumulation (Fig. 1B) of diphenhydramine were considerably decreased by lowering the pH of the apical medium.

Concentration-Dependence of Diphenhydramine Transepithelial Transport and Apical Uptake

The concentration-dependence of diphenhydramine transepithelial transport was then examined. Figure 2A shows the transepithelial transport of diphenhydramine from the apical-to-basolateral side of Caco-2 cell monolayers as a function of the diphenhydramine concentration. The initial apical-to-basolateral flux was estimated at 15 min and the curve for

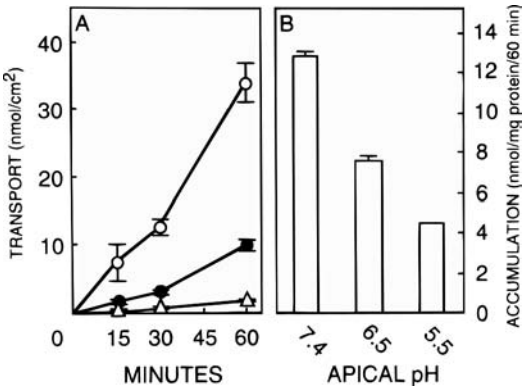

Fig. 1. Effect of apical pH on the apical-to-basolateral transport (A) and cellular accumulation (B) of diphenhydramine in Caco-2 cell monolayers. Cells grown in Transwell chambers were incubated at 37°C with 1 mM diphenhydramine (pH 7.4, ○; pH 6.5, ●; pH 5.5, △) added to the apical side, with drug-free incubation medium (pH 7.4) on the basolateral side. Appearance of diphenhydramine on the basolateral side was measured periodically. After 60 min incubation, monolayers were rapidly washed twice with 2 ml of ice-cold incubation medium on both sides, and diphenhydramine accumulation in the monolayers was determined. Each point or column represents the mean ± S.E. of five or six monolayers.

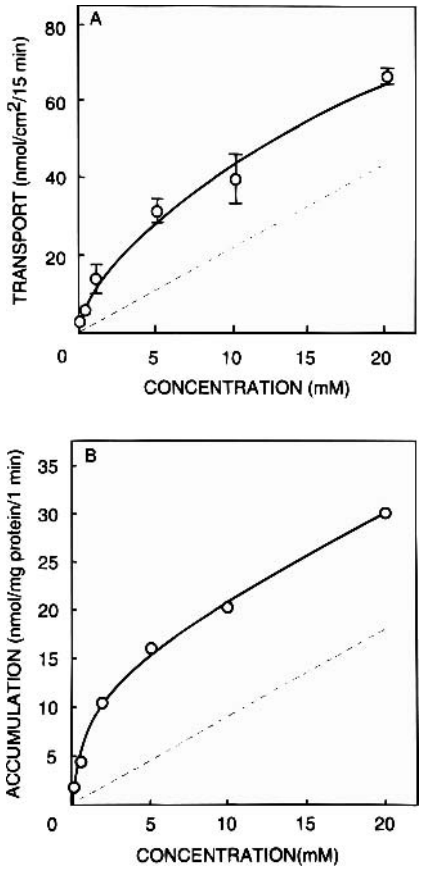

Fig. 2. Concentration-dependence of transepithelial transport (A) and initial uptake from the apical side (B) of diphenhydramine by Caco-2 cell monolayers. Cells were incubated at 37°C for 15 min (A) or 1 min (B) with incubation medium (pH 7.4) containing various concentrations of diphenhydramine added to the apical side. Appearance of diphenhydramine on the basolateral side (A) or intracellular accumulation of diphenhydramine (B) were measured. The solid and broken lines represent the total and the calculated value of nonsaturable transport, respectively. Each point represents the mean ± S.E. of three monolayers (A) or the mean of two monolayers (B) from a typical experiment.

transepithelial transport of diphenhydramine was curvilinear, indicating a saturable transport process for diphenhydramine. Kinetic parameters were evaluated using nonlinear least-squares regression analysis from the following Michaelis-Menten equation:

$$V = \frac{V\text{max}[S]}{K\text{m} + [S]} + K\text{d}[S]$$

where V is the initial accumulation rate, [S] is the initial concentration of diphenhydramine, Vmax is the maximum accumulation rate, Km is the Michaelis constant, and Kd is the coefficient of simple diffusion. The apparent Km and Vmax values for diphenhydramine transport were 1.4 mM and 24.1 nmol · mg protein^{-1} · 15 min^{-1}, respectively.

Figure 2B shows the concentration-dependence of cellular accumulation of diphenhydramine from the apical side for 1 min. Diphenhydramine uptake from the apical side was also saturable, and the data were fitted to the above equation by nonlinear least-squares regression analysis. The apparent Km and Vmax values were 0.9 ± 1.1 mM and 11.0 ± 2.0 nmol · mg protein^{-1} · min^{-1}, respectively (each value represents the mean ± S.E. of three separate experiments).

Effect of Various Organic Cations on Diphenhydramine Transport and Cellular Accumulation

The effects of various organic cations, which are transported by renal organic cation transporters, on the apical-to-basal transepithelial transport and cellular accumulation of diphenhydramine were examined. As shown in Table I, tetraethylammonium, cimetidine and guanidine had no significant effect on either transepithelial transport or cellular accumulation of diphenhydramine by Caco-2 cell monolayers. Therefore, it appears likely that the transport system for diphenhydramine is distinct from the typical organic cation/H^+ antiport system expressed in the renal brush-border membrane.

The Basolateral-to-Apical Transepithelial Transport and Cellular Accumulation of Diphenhydramine

To determine whether the transepithelial transport of diphenhydramine across Caco-2 cell monolayers is unidirectional, transepithelial flux was measured by adding diphenhydramine to either the apical or basolateral side of Caco-2 cell monolayers, and the appearance of diphenhydramine at the opposite side was examined. As shown in Fig. 3, the basolateral-to-apical transport of diphenhydramine was significantly higher than the apical-to-basolateral transport, suggesting that secretory transport of diphenhydramine is predominant rather than absorptive transport in Caco-2 cells.

We then examined the cellular accumulation of diphenhydramine from the basolateral side to determine whether a specific transport system for diphenhydramine is present in the basolateral membrane of Caco-2 cells. Similarly to diphenhydramine accumulation from the apical side, the accumulation of diphenhydramine from the basolateral side was saturable (data not shown). The apparent Km and Vmax values, evaluated according to the Michaelis-Menten equation, as described above, were 0.8 ± 1.1 mM and 7.8 ± 1.7 nmol · mg protein^{-1} · min^{-1}, respectively (each value represents the mean ± S.E. of three separate experiments). We then examined the transepithelial transport and cellular accumulation of diphenhydramine from the basolateral side as a function of pH of the basolateral

Table I. Effect of Organic Cations on Apical-to-Basolateral Diphenhydramine Transport and Cellular Accumulation by Caco-2 Cell Monolayers

Organic cations	Transport (nmol · cm^{-2} · 60 min^{-1})	Accumulation (nmol · mg protein^{-1} · 60 min^{-1})
Control	3.6 ± 0.4	3.5 ± 0.1
Tetraethylammonium	2.9 ± 0.1	3.6 ± 0.0
Cimetidine	3.7 ± 0.4	3.5 ± 0.0
Guanidine	2.7 ± 0.7	3.3 ± 0.1

Note: Caco-2 cell monolayers were incubated at 37°C for 60 min with incubation medium (pH 7.4) containing 100 μM diphenhydramine in the absence (control) or presence of the organic cations (5 mM) listed. Each value represents the mean ± S.E. of six monolayers.

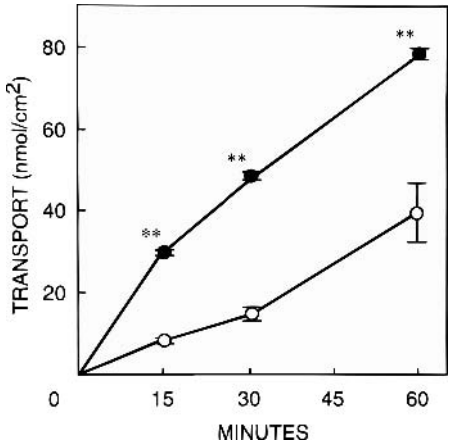

Fig. 3. Direction of transepithelial transport of diphenhydramine across Caco-2 cell monolayers. Cells were incubated with incubation medium (pH 7.4) containing 1 mM diphenhydramine added to either the apical (◯) or basolateral (●) side of monolayers. Appearance of diphenhydramine on the opposite side (pH 7.4) was measured periodically. Each point indicates the mean ± S.E. of three monolayers. ** $P < 0.01$, significantly different from apical-to-basolateral transport.

side. As shown in Fig. 4, both the basolateral-to-apical transport and cellular accumulation were affected by the pH of the basolateral side, in the order pH 7.4 > pH 6.5 > pH 5.5. When pH of the apical side was 6.0 and that of the basolateral side was 7.4, the basolateral-to-apical transport of diphenhydramine was about 13-fold greater than the apical-to-basolateral transport (data not shown), suggesting that pH-dependent transport systems could mediate the unidirectional transport of diphenhydramine in Caco-2 cell monolayers.

Efflux of Diphenhydramine from Caco-2 Cell Monolayers

Since our findings demonstrated the unidirectional transport of diphenhydramine from the basolateral side to the apical side, we next examined the efflux of diphenhydramine from Caco-2 cell monolayers. Figure 5 shows the time course of the appearance of diphenhydramine on the apical and the basolateral sides of the monolayers. The efflux rate of diphenhydramine to the apical side was much greater than that to the basolateral side. Furthermore, the efflux to the apical side was facilitated when pH of the apical side was 6.0 (Fig. 5B) as compared with pH 7.4 (Fig. 5A).

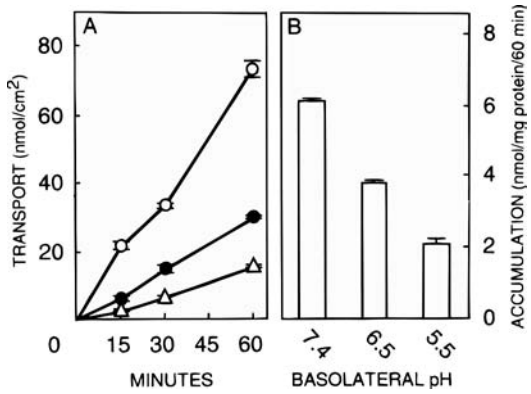

Fig. 4. Effect of basolateral pH on the basolateral-to-apical transport (A) and cellular accumulation (B) of diphenhydramine by Caco-2 cell monolayers. Cells were incubated at 37°C with 1 mM diphenhydramine (pH 7.4, ◯; pH 6.5, ●; pH 5.5, Δ) added to the basolateral side, with drug-free incubation medium (pH 7.4) on the apical side. Appearance of diphenhydramine on the apical side was measured periodically (A). After 60 min incubation, monolayers were rapidly washed twice with 2 ml of ice-cold incubation medium on both sides, and diphenhydramine accumulation in the monolayers was determined (B). Each point or column represents the mean ± S.E. of three monolayers.

Effect of Chlorpheniramine on Transepithelial Transport and Cellular Accumulation of Diphenhydramine

Previous studies have demonstrated that chlorpheniramine, another antihistamine, competitively inhibited the diphenhydramine accumulation (18). Therefore, we examined the effect of chlorpheniramine on transepithelial transport and cellular accumulation of diphenhydramine. As shown in Table II, transepithelial transport and cellular accumulation of diphenhydramine in both directions were significantly inhibited by adding an excess of chlorpheniramine (*cis*-inhibition effect). Moreover, when the monolayers were preloaded with chlorpheniramine, diphenhydramine accumulation from both sides was significantly enhanced (*trans*-stimulation effect; Table III), indicating the existence of specific transport systems for diphenhydramine and chlorpheniramine in both the apical and basolateral membranes.

DISCUSSION

Recently, we demonstrated that diphenhydramine, an antihistamine, was accumulated by the pH-dependent transport system in Caco-2 cells (18). The uptake of diphenhydramine was competitively inhibited by chlorpheniramine, whereas typical substrates for the renal organic cation transporter as well as

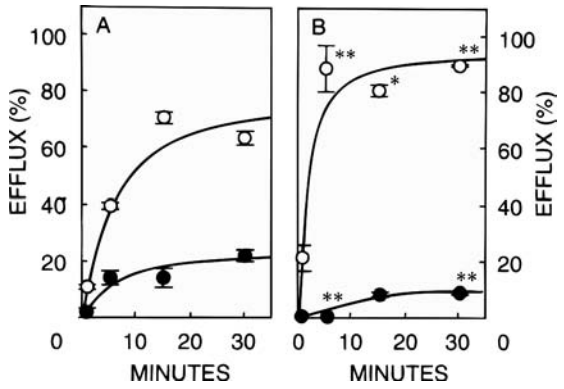

Fig. 5. Efflux of diphenhydramine from Caco-2 cell monolayers. The monolayers were incubated at 37°C for 30 min with incubation medium (pH 7.4) containing 1 mM diphenhydramine added to the basolateral side. Thereafter, the monolayers were washed twice on both sides with ice-cold incubation medium (pH 7.4). The monolayers were then incubated at 37°C with drug-free incubation medium. The medium pH levels of the apical/basolateral sides were: (A) pH 7.4/pH 7.4 or (B) pH 6.0/pH 7.4. The amounts of diphenhydramine in the apical (◯) and basolateral (●) side medium were measured. The efflux is expressed as a percentage of the cellular accumulation of diphenhydramine after 30 min preincubation. Each point represents the mean ± S.E. of three monolayers. * $P < 0.05$, ** $P < 0.01$, significantly different from respective time points in Figure 5A.

Table II. Effect of Chlorpheniramine on Diphenhydramine Transport and Cellular Accumulation by Caco-2 Cell Monolayers

Direction		Transport (nmol · cm^{-2} · 60 min^{-1})	Accumulation (nmol · mg $protein^{-1}$ · 60 min^{-1})
Apical-to-basolateral	Control	40.0 ± 6.8	10.3 ± 0.1
	Chlorpheniramine	21.1 ± 3.9	5.3 ± 0.1[a]
Basolateral-to-apical	Control	62.5 ± 4.2	7.1 ± 0.1
	Chlorpheniramine	40.2 ± 2.1[a]	3.0 ± 0.1[a]

Note: Caco-2 cell monolayers were incubated at 37°C for 60 min with incubation medium (pH 7.4) containing 1 mM diphenhydramine in the absence (control) or presence of 10 mM chlorpheniramine. Each value represents the mean ± S.E. of three monolayers.
[a] $P < 0.01$, significantly different from control.

some biological amines and neurotransmitters had no effect on diphenhydramine accumulation. To elucidate further the intestinal transport mechanism of diphenhydramine, we examined the transepithelial transport characteristics of diphenhydramine across Caco-2 cell monolayers cultured in Transwell chambers. In the present study, transepithelial transport of diphenhydramine across Caco-2 cell monolayers was shown to be decreased at lower pH (Fig. 1). This pH dependence of diphenhydramine transport might be partly explained by passive diffusion of the unionized form according to the pH-partition theory. However, diphenhydramine (pKa = 9.0) was mostly ionized even at the highest pH tested (20). Moreover, the flux and cellular accumulation of diphenhydramine were saturable (Fig. 2) and inhibited by another antihistamine, chlorpheniramine (Table II). Therefore, transepithelial transport characteristics of diphenhydramine could be explained not only by the pH-partition theory but also by the contribution of specific transport system(s).

The basolateral-to-apical transport of diphenhydramine was greater than the apical-to-basolateral transport (Fig. 3). In addition, when the extracellular pH of the apical side was more acidic than that of the basolateral side, the secretory transport of diphenhydramine was considerably facilitated. Moreover, intracellular diphenhydramine was preferentially effluxed to the apical side (Fig. 5). These findings suggest the existence of a specific secretory pathway. Indeed, intestinal secretion of various cationic drugs have been reported (2,6–8,21,22). In addition, it has been demonstrated that P-glycoprotein functions as an energy-dependent efflux pump for a variety of anti-cancer drugs and other hydrophobic compounds. P-glycoprotein is expressed in multidrug-resistant cancer cells as well as normal tissues including the gastrointestinal tract (23). Caco-2 cells also express P-glycoprotein and have been used to elucidate the intestinal secretion of various drugs (24–26). It was demonstrated that some lipophilic organic cations are actively secreted into the gastrointestinal tract via this active pump (3,10,21, 24–26). It was suggested that low bioavailability of such organic cations was partly due to secretion to the luminal side via P-glycoprotein. Indeed, diphenhydramine accumulation was enhanced under ATP-depleted conditions (Mizuuchi *et al.*, unpublished observations), suggesting the existence of the energy-dependent efflux system. Therefore, it is possible that secretory transport of diphenhydramine in Caco-2 cells might be mediated by P-glycoprotein. However, traditional antihistamines including diphenhydramine can easily permeate through the blood-brain barrier that expresses a large amount of P-glycoprotein. Furthermore, the basolateral-to-apical transepithelial transport of diphenhydramine in LLC-GA5-COL150 cells that overexpress P-glycoprotein (27,28) was not different from the host cells, LLC-PK_1 (Mizuuchi *et al.*, unpublished data). Taken together, it appears unlikely that diphenhydramine efflux is mediated by P-glycoprotein. Further studies are needed to clarify the existence of the energy-dependent secretory system.

Table III. *Trans*-Stimulation Effect of Chlorpheniramine on Diphenhydramine Uptake by Caco-2 Cell Monolayers

Uptake	Preload	Accumulation (nmol · mg $protein^{-1}$ · min^{-1})
From apical	None (Control)	15.9 ± 0.2
	Chlorpheniramine	21.5 ± 0.6[a]
From basolateral	None (Control)	11.5 ± 0.1
	Chlorpheniramine	16.2 ± 1.4[b]

Note: Caco-2 cell monolayers grown in Transwell chambers were preloaded for 30 min at 37°C with 1 mM of chlorpheniramine from both the apical and basolateral sides (pH 7.4). After removing this medium, cell monolayers were washed once with ice-cold incubation medium on both sides and then incubated for 1 min at 37°C with 5 mM of diphenhydramine on either the apical or basolateral side. Each value represents the mean ± S.E. of three monolayers.
[a] $P < 0.01$, significantly different from control.
[b] $P < 0.05$, significantly different from control.

When pH of the apical side was lowered to 6.0, both the basolateral-to-apical transepithelial transport of diphenhydramine and efflux to the apical side were significantly stimulated. These findings suggest the existence of an organic cation/H^+ antiport system in Caco-2 cells. An organic cation/H^+ antiport system is present in the renal brush-border membrane that mediates the secretion of various organic cations such as tetraethylammonium and cimetidine (11,12,29). In the present study, however, neither tetraethylammonium nor cimetidine showed inhibitory effects on diphenhydramine transport or accumulation. In contrast, the transport and accumulation of diphenhydramine were inhibited by another antihistamine, chlorpheniramine. These findings suggest that the substrate specificity of the diphenhydramine transport system is different from that of the renal organic cation/H^+ antiport system. In the small intestine, the presence of a guanidine/H^+ antiport system was reported and this transporter did not accept tetraethylammonium as a substrate (9). Nevertheless, the transport and accumulation of diphenhydramine were not inhibited by guanidine. Therefore, it appears unlikely that pH-dependent transport of diphenhydramine is mediated by the guanidine/

H^+ antiporter. Our findings suggest that a novel organic cation/H^+ antiport system might function as an absorptive barrier for diphenhydramine. However, diphenhydramine is supposed to be accumulated in Caco-2 cells via this transport system. It was reported that whether organic cations were absorbed or secreted in the intestine was dependent on the concentrations of organic cations in plasma and the intestinal lumen (7,8). Thus, this pH-dependent diphenhydramine transport system could play a pivotal role in intestinal absorption of diphenhydramine depending on its luminal concentration. Recently, it was demonstrated that the thiamine/H^+ antiport system might function as a thiamine absorption pathway in the rat intestinal brush-border membrane (30).

The mechanisms of organic cation transport in the intestinal basolateral membrane are poorly understood compared with that in the apical membrane. In the present study, the uptake of diphenhydramine from both the apical and basolateral sides was enhanced by preloading Caco-2 cell monolayers with chlorpheniramine (*trans*-stimulation effect; Table III). This finding clearly indicates the existence of specific transport systems for diphenhydramine and chlorpheniramine not only in the apical but also in the basolateral membranes of Caco-2 cells. The apparent Km value for the initial uptake from the basolateral side (0.8 mM) was almost equivalent to that from the apical side (0.9 mM). Furthermore, both the absorptive and secretory transport of diphenhydramine were affected by extracellular pH. These findings suggest that the flux of diphenhydramine is controlled by pH-dependent transport systems that exist in both the apical and basolateral membranes. Further examinations are necessary to elucidate the driving force and substrate specificity of these transport systems.

In conclusion, diphenhydramine was accumulated and transported across Caco-2 cell monolayers by pH-dependent specific transport systems that exist in both the apical and basolateral membranes. The direction of transepithelial transport and cellular efflux indicated the existence of a secretory pathway for diphenhydramine. These findings suggest that pH-dependent specific transport systems localized in both the apical and basolateral membranes are responsible for the transepithelial flux of diphenhydramine.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, and by Grants-in-Aid from the Yamada Science Foundation and the Uehara Memorial Foundation.

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