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Intestinal Brush-border Membrane Transport of Monocarboxylic Acids Mediated by Proton-coupled Transport and Anion Antiport Mechanisms

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Abstract

Intestinal brush-border membrane transport of monocarboxylic acids was investigated by using rabbit intestinal brush-border membrane vesicles (BBMVs) and isolated intestinal tissues mounted on Ussing-type chambers. [^{3}H]Mevalonic acid uptake by BBMVs showed an overshoot phenomenon in the presence of an inwardly directed proton gradient, but not in the presence of an inwardly directed sodium gradient or an outwardly directed HCO_3^- or chloride gradient. Initial uptake of mevalonic acid was saturable in the presence of a proton gradient. Uptake of [^{3}H]mevalonic acid was inhibited by various monocarboxylic acids, including acetic acid, benzoic acid, lactic acid, nicotinic acid, pravastatin, salicylic acid and valproic acid, but not by dicarboxylic acid or amino acids. Acetic acid, which is transported by both anion antiport and proton-coupled transport systems, induced serosal bicarbonate-dependent alkalinization in the mucosal-side bathing solution of rabbit jejunal tissues, when examined in Ussing-type chambers. Pravastatin, which is a structural analogue of mevalonic acid and is absorbed via proton-coupled transport like mevalonic acid, did not. The result demonstrates that acetic acid is transported by the bicarbonate-dependent anion antiport system, whereas pravastatin is not.

So, it is suggested that monocarboxylic acids are transported by at least two independent transporters, namely, a proton-coupled transporter for most monocarboxylic acids, including mevalonic acid, pravastatin and acetic acid, and an anion antiporter for acetic acid, but not for mevalonic acid or pravastatin. Activation of anion antiporter can induce HCO_3^- secretion in intact intestine.

Intestinal absorption of weak organic acids such as lactic acid (Tiruppathi et al 1988; Friedrich et al 1991, 1992), propionic acid (Harig et al 1991) and valproic acid (Allen et al 1995) has been suggested to occur by carrier-mediated mechanisms, not by passive diffusion according to the pH-partition theory (Brodie & Högben 1957). We have also suggested that several monocarboxylic acids, acetic acid (Tsuji et al 1990; Simanjuntak et al 1991), nicotinic acid (Simanjuntak et al 1990), benzoic acid (Tsuji et al 1994), salicylic acid (Takanaga et al 1994), and pravastatin (Tamai et al 1995b), permeate the intestinal brush-border membranes by carrier-mediated mechanisms (i.e. proton-coupled transport, anion-antiport mechanisms, or both) by using isolated intestinal brush-border membrane vesicles and Caco-2 cells. However, since both of the above mechanisms exhibit pH-dependent transport and have similar substrate specificity, it has not been clearly established whether the proton-coupled transporter and the anion antiporter function independently or not, namely the possibility that the proton-coupled transporter might be activated in the presence of intracellular (or intravesicular) anion (HCO_3^-) cannot be excluded.

We have demonstrated that a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, pravastatin, is transported by a proton-coupled transport mechanism, but not by an anion antiport mechanism in intestinal brush-border membrane vesicles (Tamai et al 1995b), which suggests that pravastatin could be used to discriminate the two transport mechanisms. For confirmation of the independence of the two transporters, it would be desirable to find a native substrate of the pravastatin transporter. Mevalonic acid, an intermediate in the terpenoid and cholesterol biosynthesis, is a structural analogue of pravastatin around the monocarboxylic acid moiety, and it inhibited pravastatin transport in intestinal brush-border membrane vesicles. So, it is probable that mevalonic acid could be a native substrate for the proton-coupled pravastatin transporter. In the present study, we investigated the transport mechanism of mevalonic acid and several other monocarboxylic acids by using isolated intestinal brush-border membrane vesicles in rabbits. Furthermore, in order to differentiate between the proton-coupled transporter and anion antiporter, secretion of HCO_3^- to mucosal surface induced by transport of monocarboxylic acids was examined by using rabbit jejunum, mounted on Ussing-type chamber.

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Materials and Methods

Chemicals

Mevalonolactone, (*R*)[5-^{3}H] (740 Gbq mmol^{-1}), L-[^{14}C]lactic acid (5·55 Gbq mmol^{-1}), and [^{14}C]valproic acid (2·11 Gbq mmol^{-1}) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). Mevalonic acid was prepared by alkaline hydrolysis of the mevalonolactone according to the method reported previously (Kim et al 1992). Mevalonolactone was treated with 0·05 N NaOH at room temperature for 10 min and the resultant hydrolysed solution

was adjusted to pH 7·0 with 0·1 N HCl, and stored at 4°C until use. [^{14}C]Nicotinic acid (2·03 Gbq mmol^{-1}) was purchased from Sigma Chemical Co. (St Louis, MO, USA) and [^{3}H]acetic acid (133 Gbq mmol^{-1}), and [^{14}C]benzoic acid (555 Mbq mmol^{-1}) were from New England Nuclear (Boston, MA, USA). Pravastatin and [^{14}C]pravastatin (354 Mbq mmol^{-1}) were kindly supplied by Sankyo Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade or the highest purity commercially available.

Study with intestinal brush-border membrane vesicles

The present study was performed according to the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University and was approved by the Committee on Animal Experimentation of Kanazawa University, Takara-machi Campus. Jejunal brush-border membrane vesicles from rabbit (Japan SLC, Hamamatsu, Japan) were isolated by the magnesium precipitation method as described previously (Burckhardt et al 1983) and used on the day of preparation. The resultant purified brush-border membrane vesicles were usually suspended in 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (HEPES)-Tris buffer (pH 7·5) containing 30 mM potassium gluconate and 200 mM mannitol. The uptake experiments were performed at 37°C by incubating brush-border membrane vesicles mainly in 25 mM 2-(*N*-morpholino)ethanesulphonic acid (MES)-Tris buffer (pH 5·5) containing 30 mM potassium gluconate, 10 μM valinomycin, [^{3}H]mevalonic acid and appropriate concentration of mannitol to make the solution isotonic. The amount of [^{3}H]mevalonic acid taken up by brush-border membrane vesicles was determined by measuring the radioactivity. Protein of brush-border membrane vesicles was measured by the method of Bradford (1976) by using a protein assay kit (Bio-Rad, Richmond, CA, USA) with bovine serum albumin as a standard. Uptake was represented as uptake rate (nmol (10 s)$^{-1}$ (mg protein)$^{-1}$) or vesicle/medium ratio obtained by dividing the uptake amount by the concentration of mevalonic acid in the incubation medium (μL (mg protein)$^{-1}$).

Measurement of mucosal alkalinization by the Ussing-type chamber method

Alkalinization of mucosal side of rabbit jejunum was investigated in Ussing-type chamber under a short-circuit condition by employing a pH-stat technique (Dohgen et al 1994). The isolated jejunal tissues sheets from which the external muscle layer had been removed, were mounted vertically in Ussing-type chambers that provided an exposed area of 0·5 cm^2. Bathing solutions contained no chloride throughout the study to suppress possible chloride-dependent HCO_3^- secretion in the jejunum. The serosal HCO_3^--containing solution had the following composition (in mM): sodium gluconate, 119; sodium bicarbonate, 21; calcium gluconate, 8; magnesium gluconate, 1·2; and glutamine, 2·5. This solution was gassed with 95% O_2/5% CO_2 (pH 7·4). The serosal HCO_3^--free solution had the following composition (in mM): sodium gluconate, 135; calcium gluconate, 8; magnesium gluconate, 1·2; HEPES, 10; and glutamine, 2·5. The pH value was adjusted to 7·4 with NaOH. Mucosal bathing solution had the following composition (in mM): sodium gluconate, 140; potassium gluconate, 5·4; calcium gluconate, 8; magnesium gluconate, 1·2; and mannitol, 12·5. Monocarboxylic acid was added at 25 mM as the sodium salt in place of 25 mM sodium gluconate. The pH value of the solution was adjusted to 7·4 with 0·1 M H_2SO_4 or NaOH. These HCO_3^--free solutions were gassed with 100% O_2 that had been passed through 500 mM NaOH. Mucosal alkalinization rates (J_{OH}) were determined by continuously titrating the mucosal solution to pH 7·4 with 5 mM H_2SO_4 under the automatic control of a pH-stat system (HSM-10A, Toa Electronics, Tokyo, Japan). J_{OH} in the absence of mucosal monocarboxylic acids was determined first for 1 h after an equilibration period of about 30 min. The mucosal solution was then replaced by one containing monocarboxylic acid, and after a 30 min re-equilibration period, J_{OH} was determined for 1 h. The short-circuit current (I_{SC}) was measured with the use of an automatic voltage-clamping device that compensated for the resistance between the potential-measuring electrodes, and is referred to as positive when current flowed from the mucosa to the serosa. The transepithelial conductance (G_t) was periodically determined according to Ohm's law.

Data analysis

The kinetic parameters for the uptake of mevalonic acid by brush-border membrane vesicles were estimated by solving the following equation, consisting of both saturable and apparently nonsaturable-linear terms, using the nonlinear least-squares regression analysis program MULTI (Yamaoka et al 1981):

$$v = V_{max} \times [s]/(K_m + [s]) + k_d \times [s] \quad (1)$$

where v and [s] represent the apparent uptake rate and the concentration of mevalonic acid, respectively; V_{max} and K_m are the maximum uptake rate and the apparent Michaelis constant for a carrier-mediated process, respectively, and k_d is the first-order rate constant for the apparently nonsaturable component estimated from the uptake determined at 4°C.

Results

Effect of inwardly directed proton and outwardly directed bicarbonate gradients on mevalonic acid uptake

Fig. 1 shows the time courses for the uptake of [^{3}H]mevalonic acid by brush-border membrane vesicles in the presence of proton or HCO_3^- gradients. In the presence of an HCO_3^- gradient (in the absence of a proton gradient), uptake of [^{3}H]mevalonic acid was very slow and was comparable with that in the absence of any ion gradient. In contrast, uptake of [^{3}H]mevalonic acid was accelerated in the presence of a proton gradient at an outer medium pH of 6·0 or 5·5. Moreover, [^{3}H]mevalonic acid uptake at extravesicular pH 5·5 showed a clear overshoot phenomenon. The equilibrium uptake values measured at 4 h were comparable in the presence and in the absence of a proton gradient. An inwardly directed sodium gradient did not enhance the uptake (data not shown). Although mevalonolactone is more hydrophobic than mevalonic acid, uptake of [^{3}H]mevalonic acid was faster than that of [^{3}H]mevalonolactone. In the following studies, transport was studied at extravesicular pH 5·5 when a pH gradient was present.

Concentration dependence of mevalonic acid uptake

The initial uptake of mevalonic acid was measured as a function of the substrate concentration over the range of

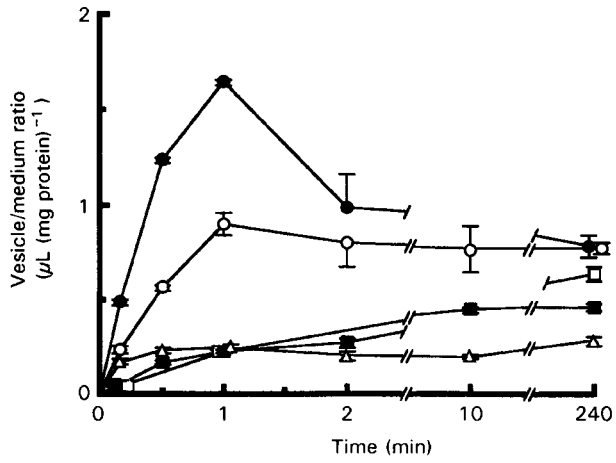

FIG. 1. Effect of pH and HCO_3^- on the uptake of [^{3}H]mevalonic acid by brush-border membrane vesicles. Brush-border membrane vesicles were preloaded with 25 mM HEPES-Tris buffer (pH 7·5) containing 30 mM potassium gluconate (○, ●, □, △) or 30 mM $KHCO_3$ (■). The uptake of [^{3}H]mevalonic acid (0·15 μM) was measured by incubating membrane vesicles in 25 mM HEPES-Tris buffer (pH 7·5, □, ■) or MES-Tris buffer (pH 5·5 ●, or pH 6·0 ○) containing 30 mM potassium gluconate. The uptake of [^{3}H]mevalonolactone (0·15 μM) was measured by incubating membrane vesicles in MES-Tris buffer (pH 5·5 △) containing 30 mM potassium gluconate.

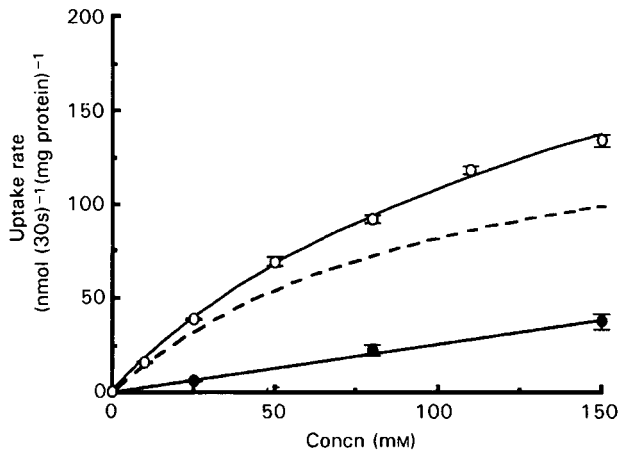

FIG. 2. Concentration dependence of mevalonic acid uptake by brush-border membrane vesicles. The uptake of mevalonic acid was measured for 30 s at 37°C (○) or 4°C (●) by incubating the membrane vesicles in 25 mM MES-Tris buffer (pH 5·5) containing 0·15 μM to 150 mM mevalonic acid and 30 mM potassium gluconate. Each point represents the mean ± s.e.m. of three to six experiments. The broken line represents a saturable uptake rate calculated using the kinetic parameters described in the text.

0·15 μM to 150 mM at 30 s in the presence of an inwardly directed proton gradient. As shown in Fig. 2, saturable uptake was observed at 37°C and the estimated V_{max}, K_m and k_d (mean ± s.d.) were 169 ± 8·1 nmol (30 s)$^{-1}$ (mg protein)$^{-1}$, 106 ± 9·7 mM and 0·249 ± 0·012 μL (30 s)$^{-1}$ (mg protein)$^{-1}$, respectively.

Counter transport effect on mevalonic acid uptake

The uptake of [^{3}H]mevalonic acid was measured for 30 s at 37°C by brush-border membrane vesicles loaded with 50 mM unlabelled mevalonic acid. Uptake of [^{3}H]mevalonic acid was significantly stimulated by preloading of unlabelled mevalonic acid from 1·30 ± 0·048 to 1·80 ± 0·025 μL (30 s)$^{-1}$ (mg protein)$^{-1}$ ($P < 0·05$ by Student's t-test).

Table 1. Inhibitory effects of various compounds on the uptake of [^{3}H]mevalonic acid.

Inhibitor	Concentration (mM)	Relative Uptake (% of control)
Acetic acid	20	87·5 ± 1·2*
Benzoic acid	20	76.2 ± 3.9*
D-Lactic acid	20	75·0 ± 0·20*
L-Lactic acid	20	75·0 ± 1·2*
Mevalonic acid	50	80·3 ± 2·6*
Nicotinic acid	20	80·1 ± 5·3*
Pravastatin	20	65·0 ± 1·4*
Salicylic acid	20	61·0 ± 1·4*
Valproic acid	20	84·4 ± 2·8*
Mevalonolactone	20	99·9 ± 8·3
Glutamic acid	20	99·9 ± 1·7
Glutamine	20	95·2 ± 2·0
Succinic acid	20	99·0 ± 4·4
FCCP	0·04	70·6 ± 1·9*
DIDS	0·1	95·7 ± 3·0

BBMVs were preloaded with 25 mM HEPES-Tris buffer (pH 7·5) containing 30 mM potassium gluconate. The uptake of [^{3}H]mevalonic acid (0·15 μM) was measured for 30 s at 37°C by incubating membrane vesicles in MES-Tris buffer (pH 5·5) containing 30 mM potassium gluconate and an indicated inhibitor. Each point represents the mean ± s.e.m. of three or four experiments. *Significantly different ($P < 0·05$) from the control.

Inhibition of [^{3}H]mevalonic acid uptake by various compounds

Table 1 shows the inhibitory effect of various compounds on the initial uptake of [^{3}H]mevalonic acid. Those structural analogues which have a carboxyl group in the molecule significantly reduced the uptake of [^{3}H]mevalonic acid. No inhibitory effect was observed with a dicarboxylic acid (succinic acid), the structural analogue mevalonolactone or amino acids (glutamic acid and glutamine). A protonophore, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), significantly reduced the uptake of [^{3}H]mevalonic acid, whereas an anion exchange inhibitor, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), was ineffective.

Uptakes of various monocarboxylic acids

Table 2 shows the initial uptake rate of several monocarboxylic acids when either an inwardly directed proton or an outwardly directed HCO_3^- gradient was imposed. The uptakes of [^{3}H]acetic acid, [^{14}C]benzoic acid, [^{14}C]nicotinic acid, L-[^{14}C]lactic acid and [^{14}C]valproic acid were greatly enhanced by imposition of either a proton or an HCO_3^- gradient compared to those in the absence of any ion gradient. On the other hand, stimulation of uptakes of [^{14}C]pravastatin and [^{3}H]mevalonic acid was observed by an imposed of proton gradient, but not by an HCO_3^- gradient.

Bicarbonate secretion accompanying monocarboxylic acid transport

To evaluate the secretion of HCO_3^- in intact tissue, alkalinization of the mucosal bathing solution was measured by using an Ussing chamber technique. Since mevalonic acid was too volatile to allow evaluation of the mucosal alkalinization caused by secretion of HCO_3^- in the present experimental

Table 2. Proton and bicarbonate dependencies of uptakes of monocarboxylic acids.

	Relative uptake	
	Proton gradient	HCO_3^--gradient
[^{3}H] Acetic acid	6·98 ± 0·44	28·7 ± 0·68
[^{14}C]Benzoic acid	5·22 ± 0·23	17·3 ± 0·14
L-[^{14}C]Lactic acid	5·16 ± 0·23	3·07 ± 0·12
[^{14}C] Nicotinic acid	7·07 ± 0·11	1·50 ± 0·15
[^{14}C]Valproic acid	8·10 ± 1·17	4·20 ± 0·32
(*R*)-[^{3}H] Mevalonic acid	3·82 ± 0·08	1·02 ± 0·11
[^{14}C] Pravastatin	13·7 ± 0·58	1·16 ± 0·09

Brush-border membrane vesicles (BBMVs) were preloaded with 25 mM HEPES-Tris buffer (pH 7·5) containing 200 mM mannitol and 30 mM potassium gluconate in the presence of proton gradient or 30 mM $KHCO_3$ in the presence of HCO_3^- gradient. The uptake of each monocarboxylic acid was measured by incubating membrane vesicles in 25 mM MES-Tris buffer (pH 6·0) in the presence of proton gradient or HEPES-Tris buffer (pH 7·5) in the presence of HCO_3^- gradient containing 200 mM mannitol and 30 mM potassium gluconate. Final concentrations of monocarboxylic acids were as follows (in μM): [^{3}H]acetic acid, 2; [^{14}C]benzoic acid, 200; L-[^{14}C]lactic acid, 7·5; [^{14}C]nicotinic acid, 50; [^{14}C]valproic acid, 100; [^{3}H]mevalonic acid, 0·15; [^{14}C]pravastatin, 200. Data represent the mean ± s.e.m. of relative uptake rate at 10 s compared with the uptake in the absence of any ion gradient (pH 7·5).

system, pravastatin was used instead of mevalonic acid, and compared with acetic acid. When rabbit jejunum was bathed on the serosal surface with an HCO_3^- (and CO_2)-containing solution, and on the mucosal surface with a monocarboxylic acid-free solution, mucosal alkalinization was observed with J_{OH} averaging 2·42 ± 0·18 μmol cm^{-2} h^{-1}, I_{sc} averaging 40·3 ± 3·9 μA cm^{-2}, and G_t averaging 13·3 ± 1·2 mS cm^{-2} (n = 11). When acetic acid was added to the mucosal side, J_{OH} was significantly increased to 3·35 ± 0·42 μmol cm^{-2} h^{-1}, as shown in Table 3. The I_{SC} and G_t values were not affected by mucosal acetic acid (39· ± 7·5 μA cm^{-2} and 14·1 ± 1·8 mS cm^{-2}, respectively). The effects of mucosal addition of pravastatin, which has been demonstrated to be transported by a proton-coupled transporter, not by an anion antiporter (Tamai et al 1995b and Table 2), on the J_{OH}, I_{SC} and G_t values were also determined. Mucosal pravastatin had no significant effect on J_{OH} (2·44 ± 0·26 μmol cm^{-2} h^{-1}, Table 3), I_{SC} (42·6 ± 3·1 μA cm^{-2}) or G_t (14·3 ± 1·1 mS cm^{-2}). In the absence of serosal HCO_3^- and CO_2, neither acetic acid nor pravastatin changed, J_{OH}, I_{SC} or G_t.

Discussion

In the present study, we first of all examined whether mevalonic acid is transported by a mechanism energized by a proton or HCO_3^- gradient across the intestinal brush-border membrane. Although imposition of an outwardly directed HCO_3^- gradient did not enhance the uptake of [^{3}H]mevalonic acid, an inwardly directed proton gradient resulted in an overshoot phenomenon (Fig. 1), saturable transport (Fig. 2) and countertransport. Furthermore, a protonophore, FCCP, reduced the initial uptake of [^{3}H]mevalonic acid, whereas an anion exchange inhibitor, DIDS, had no significant effect (Table 1). These observations suggest that mevalonic acid is transported across the intestinal brush-border membrane via a carrier-mediated transport mechanism which is energized by a proton gradient. All monocarboxylic acids examined in the present study inhibited [^{3}H]mevalonic acid uptake, but mevalonolactone, amino acids (glutamic acid and glutamine), and a dicarboxylic acid (succinic acid) did not. Succinic acid and fumaric acid (dicarboxylic acids) are transported in a sodium-dependent manner (Wolffram et al 1990, 1992), so mevalonic acid could be transported by a monocarboxylic acid-specific mechanism. Accordingly, mevalonic acid is thought to share the same proton-coupled transport mechanism with other monocarboxylic acids such as acetic acid and pravastatin.

It is noteworthy that mevalonic acid and pravastatin uptakes were not enhanced by the imposition of an outwardly directed HCO_3^- gradient which is distinct from the case with other monocarboxylic acids (Table 2). This result could be helpful in clarifying the presence of two independent transporters for monocarboxylic acids, namely an anion antiporter and a proton-coupled transporter.

The present study showed in intact jejunal mucosa that pravastatin, which were transported only by proton-coupled transport mechanism common to mevalonic acid as shown in the present and previous studies (Tamai et al 1995b), lacks the ability to secrete HCO_3^- by an anion antiport mechanism, whereas acetic acid, which is transported by both proton-coupled transport and anion antiport mechanisms, as demonstrated in Table 2 and in our previous studies (Tsuji et al 1990; Simanjuntak et al 1991), has the ability to induce mucosal HCO_3^- secretion. As shown in Table 3, acetic acid specifically increased J_{OH} in the presence, but not in the absence of serosal HCO_3^-, consistent with the finding that mucosal acetic acid can stimulate HCO_3^- secretion. Similar alkalinization

Table 3. Effect of mucosal monocarboxylic acids on the mucosal alkalinization rate.

	Luminal alkalinization rate (J_{OH}, μmol cm^{-2} h^{-1})		
	Control	+ Monocarboxylic acid	Increase in J_{OH}
Acetic acid			
+ HCO_3^-	2·51 ± 0·31	3·35 ± 0·42*	0·833 ± 0·141**
− HCO_3^-	1·41 ± 0·21	1·40 ± 0·17	− 0·012 ± 0·070
Pravastatin			
+ HCO_3^-	2·31 ± 0·21	2·44 ± 0·26	0·131 ± 0·193
− HCO_3^-	1·28 ± 0·26	1·31 ± 0·40	0·038 ± 0·160

The mucosal alkalinization rate (J_{OH}) without monocarboxylic acids was determined for the first h, then 25 mM of acetic acid or pravastatin was added to the mucosal side, and the rate was determined for another h. Each value represents the mean ± s.e.m. of five to six experiments. *Significantly different from the control value ($P < 0·05$). **Significantly different from the value in the absence of HCO_3^- ($P < 0·05$).

stimulated by acetic acid was observed in rat colon and was ascribed to the monocarboxylic acid-mediated secretion of HCO_3^- from the serosal side (Umesaki et al 1979; Dohgen et al 1994). In contrast to acetic acid, pravastatin did not change J_{OH} either in the presence or absence of serosal HCO_3^- indicating it can hardly stimulate HCO_3^- secretion. Although the proton-coupled transporter should cause mucosal alkalinization by transferring protons from the lumen to the intracellular milieu, pravastatin did not cause such an alkalinization. This could be explained by an inefficient transport activity of the proton-coupled transporter at neutral pH, simultaneous proton secretion from the epithelial cells by the Na^+/H^+ exchanger, or both.

In conclusion, the findings on differential substrate specificity and ability to cause HCO_3^- movement indicate that two independent transporters, a proton-coupled transporter and an anion antiporter, function for the intestinal absorption of monocarboxylic acids. Recently, molecular studies on monocarboxylic acid transport have been reported, including cDNA cloning of the proton-coupled monocarboxylate transporter from rat intestine (Tamai et al 1995a; Takanaga et al 1995) and from rat skeletal muscle (Jackson et al., 1995) and purification of a lactate transporter protein from erythrocytes (Poole & Halestrap 1994). These lines of study will help in understanding the multiplicity and classification of the membrane transporters of monocarboxylic acid compounds.

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