Participation of Monocarboxylic Anion and Bicarbonate Exchange System for the Transport of Acetic Acid and Monocarboxylic Acid Drugs in the Small Intestinal Brush-Border Membrane Vesicles

Matheus Timbul SIMANJUNTAK, Tetsuya TERASAKI, Ikumi TAMAI, and Akira TSUJI*

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi 13-1, Kanazawa 920, Japan

(Received February 22, 1991)

A participation of bicarbonate exchange system for the transport of acetic acid and the related monocarboxylic acid drugs in the intestinal brush-border membrane vesicles (BBMV_s) was investigated. The uptake of [³H]acetic acid at 37 °C by BBMV_s was markedly stimulated and showed a clear overshoot phenomenon in the presence of outward-directed bicarbonate gradient ($\text{pH}_{\text{in}} = 7.5$, $[\text{KHCO}_3]_{\text{in}}$ or $[\text{NaHCO}_3]_{\text{in}} = 100 \text{ mM}$; $\text{pH}_{\text{out}} = 7.5$, $[\text{K-gluconate}]_{\text{out}}$ or $[\text{Na-gluconate}]_{\text{out}} = 100 \text{ mM}$). This uptake process was saturable ($K_t = 50.4 \pm 4.96 \text{ mM}$ and $J_{\text{max}} = 11.6 \pm 0.61 \text{ nmol/mg protein/10 s}$) and was inhibited by DIDS (4,4-diisothiocyano-2,2'-disulfonic acid stilbene disodium salt) and furosemide, anion exchange inhibitors, and by many monocarboxylates. The initial uptake of [³H]acetic acid was competitively inhibited by salicylic acid, suggesting the common transport between acetic acid and salicylic acid. At lower extravascular pHs and in the presence of outward-directed bicarbonate gradient ($\text{pH}_{\text{in}} = 7.5$, $[\text{KHCO}_3]_{\text{in}} = 100 \text{ mM}$; $\text{pH}_{\text{out}} = 6.0$ or 5.0 , $[\text{K-gluconate}]_{\text{out}} = 100 \text{ mM}$) where membrane potential was clamped to zero by K^+ -valinomycin, the uptake of [³H]acetic acid showed an overshoot phenomenon, whereas the uptake was significantly decreased in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, a protonophore. It was concluded, therefore, that there are one or two mechanisms for the carrier-mediated transport of acetic acid and monocarboxylates related to bicarbonate exchange systems in rabbit intestinal BBMV_s: 1) proton gradient independent and bicarbonate exchange system; 2) proton gradient dependent and bicarbonate exchange system.

Keywords — intestinal uptake; brush-border membrane vesicle; carrier-mediated transport; bicarbonate exchange system; active transport; acetic acid; monocarboxylic acid; salicylic acid

Introduction

In our previous studies, we have demonstrated that pH-dependent intestinal brush-border membrane transport of nicotinic acid and short chain fatty acids (SCFA) can be explained by carrier-mediated and H^+ -cotransport mechanism^{1,2)} instead of the passive diffusion mechanism according to the previously defined pH-partition hypothesis.³⁻⁵⁾ In addition to this transport system, most recently, Titus and Ahearn⁶⁾ proposed a mechanism for SCFA transport in the small intestine of a herbivorous teleost that there is a specific exchange system for anions that is shared by bicarbonate and SCFA. It has been found in some animals and in man that intestinal absorption of SCFA is accompanied by the luminal appearance of bicar-

bonate.⁷⁾ The two mechanisms responsible for this phenomenon have been proposed: 1) a non-mediated and passive diffusion preceded by intraluminal protonation of SCFA anions⁷⁾ and 2) a carrier-mediated transport by $\text{SCFA}^-/\text{HCO}_3^-$ exchange system.⁷⁻¹⁰⁾ However, the mechanism by which luminal bicarbonate appearance and SCFA disappearance are coupled has not yet been clearly identified.

This study was undertaken, for the uptake of [³H]acetic acid by rabbit intestinal brush-border membrane vesicles (BBMV_s), to clarify a participation of $\text{SCFA}^-/\text{HCO}_3^-$ exchange system and the relation to the monocarboxylic acid drugs in addition to the previously found H^+ -cotransport system.

* To whom correspondence should be addressed.

Materials and Methods

Materials — [^3H]Acetic acid (3.6 Ci/mmol) was purchased from Amersham International, Buckinghamshire, U.K. Valinomycin, 4, 4'-diisothiocyano-2,2'-disulfonic acid stilbene, disodium salt (DIDS) and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Unlabeled acetic acid, propionic acid, butyric acid, valproic acid, salicylic acid, benzoic acid, nicotinic acid, L(+)-lactic acid, D(-)-lactic acid, and furosemide were purchased from Wako Pure Chemical Industries, Ltd., (Tokyo, Japan). All other chemicals were of reagent grade and used without further purification.

Preparation of the Rabbit Intestinal BBMVs — BBMV's were isolated and purified from the small intestine of male rabbits weighing 1.5–2.0 kg (Sankyo Laboratory Co., Toyama, Japan), by the method of Kessler *et al.*¹¹⁾ with a slight modification described previously.¹²⁾ The final membrane vesicles were suspended in the medium of 10 mM Tris (2-amino-2-hydroxymethyl-1,3-propanediol)-Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, at pH 7.5 containing 100 mM mannitol and 100 mM NaHCO_3 or KHCO_3 . To minimize effects of proton gradient for the membrane transport, 20 μM FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), a protonophore, was added into the medium.¹³⁾ The purity of the vesicles was assessed by measurement of the specific activity of alkaline phosphatase¹⁴⁾ and the value of relative enzyme activity (suspension of membrane vesicles/homogenate of small intestine) was enriched to be 12–14 times (from 12 independent experiments).

Uptake Experiments — The uptake of [^3H]acetic acid by BBMV's was performed at 37 °C by a rapid filtration method described previously by Tsuji *et al.*¹²⁾ Membrane vesicles and the uptake medium were preincubated for 5 min at 37 °C before the initiation of the uptake experiment. The reaction was initiated by adding, to 10 μl of membrane vesicles, 90 μl aliquots of the uptake medium containing 100 mM mannitol and 100 mM Na-gluconate or K-gluconate, 10

mM Tris-Hepes buffer (pH 7.5) or 10 mM Tris-Mes (2-(*N*-morpholino)ethanesulfonic acid) (pH 6.5, 6.0 or 5.0), 20 μM FCCP, [^3H]acetic acid (5.39 μM as the final concentration) and the other constituents. The uptake reaction was carried out at 37 °C and terminated at the desired time by an addition of 1 ml of ice-cold stop solution (100 mM mannitol, 100 mM NaHCO_3 or KHCO_3 and 10 mM Tris-Hepes, pH 7.5). The diluted samples were immediately applied on a millipore filter (HAWP, 0.45 μm , Nihon Millipore Ltd., Tokyo, Japan) and rapidly washed twice with 4 ml of ice-cold stop solution. Radioactivity was counted in a liquid scintillation counter (Model LSC-700 liquid scintillation counter, Aloka Co., Tokyo, Japan). Non-specific binding of the substrate to the filter was determined by filtering the reaction mixture without vesicles. Proteins were measured by the method of Bradford¹⁵⁾ by using a Bio-Rad protein assay kit (Richmond, CA, U.S.A.) with bovine serum albumin as a standard.

Results

The present uptake study was carried out at 37 °C so that a reliable uptake rate could be obtained, although our previous study¹⁾ on [^3H]acetic acid uptake by rabbit intestinal BBMV's has been performed at 27 °C because of the rapid uptake at 37 °C in the presence of the inward-directed proton gradient. Except for the experiments examining the extravesicular pH effect, 20 μM of FCCP, a protonophore which dissipates rapidly the proton gradient across the biological and artificial lipid membrane, was added to the uptake medium to minimize proton effects on membrane vesicles.

Effect of Osmolarity on [^3H]Acetic Acid Uptake

The intravesicular size responds to the changes of the osmolarity in the extravesicular medium. Therefore, in order to examine whether the apparent uptake of acetic acid was caused by a binding to the membrane surface or an intravesicular accumulation, the steady state uptake of [^3H]acetic acid was measured at 5 min by increasing the osmolarity of the outer medium with mannitol at the concentration range of

Fig. 1. Effect of Osmolarity on [³H]Acetic Acid Uptake
Intestinal BBMV's were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM NaHCO₃ and 100 mM mannitol. The uptake of [³H]acetic acid (5.39 μM) was measured for 5 min at 37 °C by incubating membrane vesicles in 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM Na-gluconate and mannitol of various concentrations (270–820 mM). Each point represents mean ± S.E. of three to four experiments.

Fig. 2. Effect of Extravesicular Anion on [³H]Acetic Acid Uptake

For control uptake, intestinal BBMV's were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM Na-gluconate and 100 mM mannitol. The control uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C by incubating in 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM Na-gluconate and 100 mM mannitol (closed triangle). For evaluating the intravesicular bicarbonate and the extravesicular anion effects, BBMV's were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM NaHCO₃ and 100 mM mannitol, then the uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C by incubating in 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM mannitol and 100 mM Na-gluconate (open circles) or 100 mM NaCl (closed circles) and 100 mM Na-gluconate with 0.5 mM acetic acid (closed squares). The final concentrations of FCCP, a protonophore, and ethanol were 20 μM and 0.5%, respectively. Each point represents the mean ± S.E. of three to four experiments.

270 to 820 mM. As shown in Fig. 1, the steady-state uptake of [³H]acetic acid decreased significantly with the increase in mannitol concentration, indicating that [³H]acetic acid is taken up into an osmotically sensitive vesicular space. The value obtained from the extrapolation of the mannitol concentration to the infinity suggests that the amount of surface binding was about 20% of the equilibrium uptake.

Effects of Outward-Directed Bicarbonate Gradient and Extravesicular Anions on [³H]Acetic Acid Uptake

The effects of outward-directed bicarbonate gradient and extravesicular anions have been examined for [³H]acetic acid uptake by the rabbit intestinal BBMV's. As illustrated in Fig. 2, in the absence of proton and bicarbonate gradients (pH_{in} = pH_{out} = 7.5; [NaHCO₃]_{in} = [NaHCO₃]_{out} = 0 mM), [³H]acetic acid uptake

TABLE I. Inhibition of [³H]Acetic Acid Uptake by Various Compounds

Inhibitor	Concentration (mM)	% of uptake ^{a)}
Control		100
Acetamide	100	100.1 ± 6.36 (3)
Acetic acid	100	29.5 ± 4.21 ^{b)} (4)
Propionic acid	100	38.3 ± 3.40 ^{b)} (4)
Butyric acid	100	52.8 ± 8.19 ^{b)} (3)
Valproic acid	100	67.0 ± 6.52 ^{b)} (4)
Benzoic acid	100	43.5 ± 3.24 ^{b)} (3)
Salicylic acid	100	19.2 ± 2.91 ^{b)} (3)
Nicotinic acid	100	21.2 ± 5.04 ^{b)} (3)
Propicillin K	100	12.9 ± 3.14 ^{b)} (3)
L(+)-Lactic acid	100	51.8 ± 13.8 ^{b)} (3)
D(-)-Lactic acid	100	89.9 ± 16.9 (3)
DIDS	1	14.9 ± 11.6 ^{b)} (3)
Furosemide	1	20.2 ± 6.22 ^{b)} (3)

a) Intestinal BBMV's were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM NaHCO₃ and 100 mM mannitol. The uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C by incubating in 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM Na-gluconate and 100 mM mannitol and indicated inhibitor. The final concentrations of FCCP, a protonophore, and ethanol were 20 μM and 0.5%, respectively. Each value represents the mean ± S.E.; the number of experiments is shown in parentheses.
b) Significantly different when compared with the control uptake (*p* < 0.05).

was very slow. In contrast, [^3H]acetic acid uptake was accelerated by many-fold in the presence of outward-directed bicarbonate gradient and absence of proton gradient ($\text{pH}_{\text{in}} = \text{pH}_{\text{out}} = 7.5$; $[\text{NaHCO}_3]_{\text{in}} = 100 \text{ mM}$, $[\text{Na-gluconate}]_{\text{out}} = 100 \text{ mM}$). For example, the initial uptake rates measured with 10 s incubation were 3-fold enhanced. Moreover, the time course of [^3H]acetic acid uptake in the presence of bicarbonate gradient showed a clear overshoot phenomenon, indicating transient accumulation of acetate inside the vesicles against a concentration gradient. However, the equilibrium values measured with 5 min incubation were similar in the presence and absence of bicarbonate gradient. Another noteworthy observation is that the uptake of [^3H]acetic acid was almost the same as compared to those in which the composition of outer medium was changed from 100 mM Na-gluconate to 100 mM NaCl or 100 mM Na-gluconate containing 0.5 mM unlabelled acetic acid, at which acetic acid concentration the H^+ cotransport of [^3H]acetic acid was found to be inhibited completely.¹⁾

Inhibition of [^3H]Acetic Acid Uptake by Various Compounds

Table I shows the inhibitory effects of various compounds on the initial uptake of [^3H]acetic acid. The structural analogues such as monocarboxylic acids, *i.e.*, aliphatic monocarboxylic acids (propionic acid, butyric acid, valproic acid, L(+)-lactic acid) and aromatic monocarboxylic acids (salicylic acid, benzoic acid, nicotinic acid, propicillin K) and also DIDS and furosemide, anion exchange inhibitors, reduced significantly the uptake of [^3H]acetic acid. On the contrary, there was no inhibitory effect by acetamide and D(-) lactic acid.

Lineweaver-Burk Plots for Inhibitory Effect of Salicylic Acid on pH Independent [^3H]Acetic Acid Uptake

The inhibitory effect of 50 mM salicylic acid on the uptake of acetic acid (30 mM–100 mM) at 10 s was kinetically studied at 37 °C in the absence of proton gradient but in the presence of outward-directed bicarbonate gradient. The saturable uptake was corrected for the non-saturable component in total transport by subtracting the value of $28.7 \pm 3.8 \text{ pmol/mg protein/10 s/mM}$ which was determined at acetic acid concentration of 100 mM and at 4 °C. Figure 3 shows the Lineweaver-Burk plots for

Fig. 3. Lineweaver-Burk Plots of [^3H]Acetic Acid Uptake

The condition for preloading BBMV was the same as described in Fig. 1. The uptake of [^3H]acetic acid was performed at 37 °C by incubating in 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM Na-gluconate in the absence of (control, open circles) or in the presence 50 mM salicylic acid (closed circles). All experiments were performed in the presence of 20 μM FCCP, a protonophore, and 0.5% ethanol. The values are corrected for the non-saturable uptake (see text). Each point represents the mean $\pm$ S.E. of three to four experiments.

Fig. 4. Effect of Extravesicular pH for the Uptake of [³H]Acetic Acid

Intestinal BBMVs were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM KHCO₃ and 100 mM mannitol. The uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C and for various times by incubating with 100 mM mannitol and 100 mM K-gluconate in 10 mM Tris-Hepes buffer (pH 7.5, open circles) or with 10 mM Tris-Mes buffer (pH 6.0, closed squares), or with 10 mM Tris-Mes buffer (pH 5.0, closed circles), or with 10 mM Tris-Mes buffer and 20 μM FCCP (pH 5.0, closed triangles). All experiments were performed in the medium containing 2.0 μM valinomycin, a K⁺-ionophore, and 0.5% ethanol. Each point represents the mean ± S.E. of three to four experiments.

Fig. 5. pH Dependence of [³H]Acetic Acid Uptake

Intestinal BBMVs were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 100 mM KHCO₃ and 100 mM mannitol. The uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C for 10 s by incubating in 10 mM Tris-Hepes (pH 7.5) or 10 mM Tris-Mes (pH 6.5) or 10 mM Tris-Mes (pH 6.0) containing 100 mM KHCO₃, 100 mM mannitol and 0.5 mM acetic acid. All experiments were performed in the medium containing 2.0 μM valinomycin, a K⁺-ionophore, and 0.5% ethanol. Each column represents the mean ± S.E. of three to four experiments.

saturable uptake rates of acetic acid. The result demonstrates that salicylic acid inhibited competitively the uptake of acetic acid and therefore was fitted to Eq. 1 using a nonlinear least squares regression analysis program, MULTI.¹⁶⁾

$$J = J_{\max} S / [K_t (1 + I/K_i) + S] \quad (1)$$

where J is the saturable uptake rate, S is acetic acid concentration in the uptake medium, and I is salicylic acid concentration used as an inhibitor, $J_{\max}$ and K_t are the maximum uptake rate and Michaelis constant for acetic acid transport and K_i is the inhibitory constant of salicylic acid. The values of K_t , $J_{\max}$ and K_i thus estimated were 50.4 ± 4.96 mM, 11.6 ± 0.61 nmol/mg protein/10 s, and 18.7 ± 4.70 mM, respectively.

Effect of Extravesicular pH for [³H]Acetic Acid Uptake

In order to determine the effect of inward-directed proton gradient for the exchange uptake of [³H]acetic acid with bicarbonate ion, the

time courses of the uptake were measured at the different extravesicular pHs under the voltage-clamped condition produced by valinomycin, where membrane potential was clamped to zero. The results are given in Fig. 4, showing that the initial rates of the uptake measured at 10 s were stimulated in the presence of inward-directed proton gradient and outward-directed bicarbonate gradient (pH_{in} = 7.5, [KHCO₃]_{in} = 100 mM; pH_{out} = 6.0 or 5.0, [K-gluconate]_{out} = 100 mM) as compared to those in the absence of proton gradient and the presence of outward-directed bicarbonate gradient (pH_{in} = 7.5, [KHCO₃]_{in} = 100 mM; pH_{out} = 7.5, [K-gluconate]_{out} = 100 mM), while the equilibrium values were the same in both cases. The other important finding is that 20 μM FCCP, a protonophore, reduced significantly the uptake of [³H]acetic acid in the presence of inward-directed proton gradient and outward-directed bicarbonate gradient.

When compared with the data shown in Figs. 4 and 5, in the absence of bicarbonate gradient

Fig. 6. Effect of Intravesicular Bicarbonate Concentration on [³H]Acetic Acid Uptake

Intestinal BBMVs were preloaded with 10 mM Tris-Hepes buffer (pH 7.5) containing 25, 50, 75 and 100 mM NaHCO₃. To adjust the osmolarity at 300 mOsm, mannitol was added to the preloading medium at 250, 200, 150 and 100 mM. The uptake of [³H]acetic acid (5.39 μM) was performed at 37 °C for 10 s by incubating in 10 mM Tris-Mes buffer (pH 6.0) containing 25, 50, 75 and 100 mM Na-gluconate. Mannitol was also added to the uptake medium at 250, 200, 150 and 100 mM to adjust the osmolarity at 300 mOsm. Each column represents the mean ± S.E. of three to four experiments.

and proton-gradient ($[\text{KHCO}_3]_{\text{in}} = [\text{KHCO}_3]_{\text{out}} = 100 \text{ mM}$; $\text{pH}_{\text{in}} = \text{pH}_{\text{out}} = 7.5$), the uptake was reduced about 10-fold compared to the uptake in the presence of outward-directed bicarbonate gradient because of *cis*-inhibition by bicarbonate in the outside vesicles. Under this condition of the absence of bicarbonate gradient, however, as clearly seen in Fig. 5, the uptake of [³H]acetic acid was accelerated by 3–15 fold in the presence of inward-directed proton gradient, which was significantly reduced by FCCP, although the effect of FCCP was incomplete.

Effect of Intravesicular Bicarbonate Concentration on [³H]Acetic Acid Uptake

The uptake of [³H]acetic acid was measured as a function of varying intravesicular bicarbonate concentrations under inward-directed proton gradient ($\text{pH}_{\text{in}} = 6.0$, $\text{pH}_{\text{out}} = 7.4$). It can be seen from Fig. 6 that the greater intravesicular bicarbonate concentration lead to increases in [³H]acetic acid uptake, although the uptake was minimum at 50 mM intravesicular bicarbonate concentration. Under this condition,

acetic acid uptake was also saturable with the saturation half concentration about 3 mM (data not shown).

Discussion

The present results demonstrate that in addition to a H⁺-cotransport system as found in our laboratory,¹⁾ acetic acid can be transported in an osmolarity sensitive manner *via* SCFA⁻/HCO₃⁻ exchange system in the rabbit intestinal brush-border membrane. Since DIDS and furosemide, anion exchange inhibitors, reduced significantly the uptake of [³H]acetic acid in the presence of an outward-directed bicarbonate gradient (Table I), it was strongly indicated that there is an anion exchange system in the intestinal acetic acid transport. The results obtained in this study are very similar to those reported in the small intestine of herbivorous teleost⁶⁾ and in human colon,¹⁰⁾ where the uptakes of acetic acid and propionic acid by the membrane vesicles isolated from both species have been demonstrated to proceed *via* a bicarbonate exchange system. No significant inhibition by 100 mM chloride anion on [³H]acetic acid uptake in the presence of an outward-directed bicarbonate gradient (Fig. 2) suggests that this bicarbonate exchange system is different from Cl⁻/HCO₃⁻ exchange system which has been well established by the studies using intestinal BBMVs.¹⁷⁾

Another important aspect is that SCFA⁻/HCO₃⁻ exchange system and the previously found H⁺-cotransport system for acetic acid uptake by rabbit intestinal BBMVs are independent of each other. This aspect was supported by the data obtained from Fig. 2, since 0.5 mM unlabelled acetic acid, which reduced completely the uptake of [³H]acetic acid by H⁺-cotransport system,¹⁾ had no effect on [³H]acetic acid uptake in the presence of an outward-directed bicarbonate gradient and in the absence of a proton gradient. This is also supported by the fact shown in Fig. 3, *i. e.*, $J_{\text{max}} = 11.6 \text{ nmol/mg protein/10 s}$ at 37 °C for SCFA⁻/HCO₃⁻ exchange system is about 10⁴ times greater than $J_{\text{max}} = 1.62 \text{ pmol/mg protein/10 s}$ at 27 °C for the H⁺-cotransport sys-

tem in the rabbit intestinal brush-border membrane for acetic acid reported previously.¹⁾ This large difference in $J_{\max}$ values cannot be explained by the difference in the temperature performed between the present (37 °C) and the previous (27 °C) studies.¹⁾

A participation of a carrier-mediated transport system in acetic acid transport across the intestinal brush-border membrane is further supported by the finding in Table I, which shows the inhibitory effects of various compounds on the initial uptake of [³H]acetic acid in the presence of an outward-directed bicarbonate gradient but in the absence of a proton gradient. All monocarboxylic acid derivatives such as propionic acid, butyric acid, salicylic acid, benzoic acid, nicotinic acid, L(+)lactic acid and propicillin as well as the unlabelled acetic acid at the concentration of 100 mM reduced significantly the uptake. However, acetamide and D(-)lactic acid at the concentration of 100 mM had no effect. Especially, the stereospecific inhibition by lactic acid indicates strongly the carrier-mediated uptake of acetic acid.

In addition to the above inhibitory effects observed only by monocarboxylic acids, the result (Fig. 3) showing the competitive inhibition by salicylic acid for [³H]acetic acid uptake indicates the common transport system between acetic acid and monocarboxylates.

Besides the SCFA⁻/HCO₃⁻ exchange system, a participation of an inward-directed proton gradient dependent bicarbonate exchange system for [³H]acetic acid could be postulated. This transport system was supposed by the results shown in Fig. 4, which shows that at low pH_{out} and in the absence of a bicarbonate gradient, [³H]acetic acid uptake showed an overshoot phenomenon, whereas the uptake was decreased in the presence of 20 μM FCCP. This proton dependent and bicarbonate exchange system appeared to be different from the H⁺-cotransport system demonstrated in the previous study,¹⁾ because the proton gradient dependent uptake was still observed in spite of the presence of 0.5 mM acetic acid in the medium (Fig. 5). These results can be explained by the participation of two transport routes relating the SCFA⁻/HCO₃⁻ exchange system on

[³H]acetic acid transport across the intestinal brush-border membrane. These are as follows: 1) SCFA⁻/HCO₃⁻ exchange with a proton dependence; 2) SCFA⁻/HCO₃⁻ exchange with a proton independence.

The role of a proton gradient as the driving force for bicarbonate exchange transport of acetic acid in small intestine has physiological significance. In small intestine, the pH of the close vicinity of the brush-border membrane is reported to be acidic compared with the pH of the luminal solution.¹⁸⁾ This microclimate pH has been determined to be in the range of 5.5–6.0.¹⁸⁾ But the intracellular pH of intestinal epithelial cells is approximately 7.0.¹⁹⁾ Therefore, it is obvious that an inward-directed proton gradient across the small intestinal brush-border membrane exists *in vivo*. As understood from the results shown in Figs. 5 and 6, this proton gradient may provide the driving force for the intestinal absorption of acetic acid in the presence of bicarbonate both at outside and inside of the brush-border membrane. This proton dependent uptake rate of acetic acid may be accelerated in the presence of an outward-directed bicarbonate gradient.

In conclusion, the previously found H⁺-cotransport system, acetic acid and the related monocarboxylic acid drugs were demonstrated to be transported across the small intestinal brush-border membrane by an SCFA⁻/HCO₃⁻ exchange system with a proton gradient independent manner and in part with proton gradient dependent manner. The presently found mechanism would explain the previously unsolved phenomena exhibiting the intestinal SCFA absorption coupled by the luminal secretion of bicarbonate.

Acknowledgements This work was taken in part from a dissertation submitted by Matheus Timbul Simanjuntak to the Graduate School of Natural Science and Technology, Division of Life Sciences, Kanazawa University, Kanazawa, Japan, in partial fulfillment of the Doctor of Philosophy degree requirements.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan,

the Project Research Foundation of the Graduate School of Natural Science and Technology, Kanazawa University and the Uehara Memorial Foundation. One of the authors (M. T. S) thanks the Ministry of Education, Science and Culture, Japan, for their scholarship support for him. Authors also thank Mr. Atsushi Kadowaki for assistance in the preparation of the manuscript.

References

- 1) A. Tsuji, M. T. Simanjuntak, I. Tamai, and T. Terasaki: pH-Dependent intestinal transport of monocarboxylic acids: Carrier-mediated and H^+ -cotransport mechanism *versus* pH-partition hypothesis, *J. Pharm. Sci.*, **79**, 1123—1124 (1990).
- 2) M. T. Simanjuntak, I. Tamai, T. Terasaki, and A. Tsuji: Carrier-mediated uptake of nicotinic acid by rat intestinal brush-border membrane vesicles and relation to monocarboxylic acid transport, *J. Pharmacobio-Dyn.*, **13**, 301—309 (1990).
- 3) J. Elbert, H. Daniel, and G. Rehner: Intestinal uptake of nicotinic acid as a function of microclimate-pH, *J. Vit. Nutr. Res.*, **56**, 85—93 (1986).
- 4) T. T. Kararli: Gastrointestinal absorption of drugs, *J. Critical reviews in therapeutic drug carrier system*, **6**, 39—86 (1989).
- 5) A. J. M. Watson, E. A. Brennan, M. J. G. Farthing, and P. D. Fairclough: Acetate uptake by intestinal brush border membrane vesicles, *Gut*, **32**, 383—385 (1991).
- 6) E. Titus and G. A. Ahearn: Short-chain fatty acid transport in the intestine of a herbivorous teleost, *J. Exp. Biol.*, **135**, 77—94 (1988).
- 7) M. Bugaut: Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals, *Comp. Biochem. Physiol.*, **86B**, 439—472 (1987). References are cited in.
- 8) M. G. Schmitt, Jr., K. H. Soergel, C. M. Wood, and J. J. Steff: Absorption of short-chain fatty acids from the human ileum, *Dig. Dis.*, **22**, 340—347 (1977).
- 9) Y. Umesaki, T. Yajima, K. Tohyama, and M. Mutai: Characterization of acetate uptake by the colonic epithelial cells of the rat, *Pfugers Arch.*, **388**, 205—209 (1980).
- 10) K. H. Soergel, J. M. Harig, F. D. Loo, K. Ramaswamy, and C. M. Wood: Colonic fermentation and absorption of SCFA in man, *Acta Vet. Scand. Suppl.*, **86**, 107—115 (1989).
- 11) M. Kessler, O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza: A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush-border membrane, *Biochim. Biophys. Acta*, **506**, 136—154 (1978).
- 12) A. Tsuji, T. Terasaki, I. Tamai, and H. Hirooka: H^+ gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across brush-border membrane vesicles from rat small intestine, *J. Pharmacol. Exp. Ther.*, **241**, 594—601 (1987).
- 13) N. Takuwa, T. Shimada, H. Matsumoto, M. Himukai, and T. Hoshi: Effect of hydrogen ion-gradient on carrier-mediated transport of glycylglycine across brush border membrane vesicles from rabbit small intestine, *Jpn. J. Physiol.*, **35**, 629—642 (1985).
- 14) K. Walter and C. Schutt: Acid and alkaline phosphatase in serum, "Methods of Enzymic Analysis," Vol. II, ed. by H. U. Bergmeyer, Academic Press, New York, 1974, pp. 856—864.
- 15) M. M. Bradford: Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.*, **72**, 248—254 (1976).
- 16) K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno: Pharmacokinetic analysis program (MULTI) for microcomputer, *J. Pharmacobio-Dyn.*, **4**, 879—885 (1981).
- 17) R. Knickelbein, P. S. Aronson, C. M. Schron, J. Seifter, and J. W. Dobbins: Sodium and chloride transport across rabbit ileal brush-border II. Evidence for $Cl^- - HCO_3^-$ exchange and mechanism of coupling, *Am. J. Physiol.*, **249**, G236—G245 (1985).
- 18) M. L. Lucas, W. Schneider, F. J. Haberich, and J. A. Blair: Direct measurement by pH-microelectrode of the pH microclimate in rat proximal jejunum, *Proc. R. Soc. London B.*, **192**, 39—48 (1975).
- 19) P. Kurtin and A. N. Charney: Intestinal ion transport and intracellular pH during acute respiratory alkalosis and acidosis, *Am. J. Physiol.*, **247**, G24—G31 (1984).