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The transport mechanisms of organic cations and their zwitterionic derivatives across rat intestinal brush-border membrane. II. Comparison of the membrane potential effect on the uptake by membrane vesicles

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Further investigation of organic cation transport mechanisms were continued using rat intestinal brush-border membranes following our previous report [1,2]. The net uptake of organic cations was superior to that of their zwitterionic derivatives. This result agreed with the absorption behaviour of these compounds from rat intestinal loop. The uptake of tyramine and 5-benzyloxytryptamine was significantly stimulated by the valinomycin-generated K^+-diffusion potential (inside-negative). On the other hand, the uptake of zwitterionic derivatives was not affected by the valinomycin-induced K^+-diffusion potential. The voltage-clamped brush-border membrane vesicles exhibited a complete disappearance of the overshoot-uptake of organic cations. Therefore, this permeation mechanism across the intestinal brush-border membrane seems to be different from the well-known H^+-antiport system of organic cation found in other organs such as kidney and liver, and depends upon an inside-negative H^+- or K^+-diffusion potential.

Introduction

In our previous paper [1], the bindings of organic cations, such as tyramine, tryptamine, and 5-benzyloxytryptamine (BOTA), were demonstrated to be remarkably higher than those of their zwitterionic derivatives in both intestinal brush-border membrane vesicles and liposome containing phosphatidylserine. The binding of organic cations to the liposome was increased proportionally to the content of phosphatidylserine, an acidic phospholipid, and the double-reciprocal plot of tryptamine binding to the liposome with BOTA resulted in a competitive inhibition which was similar to the result of tryptamine uptake by brush-border membrane vesicles in another paper from our group [2].

On the other hand, we have reported that the effect of the ionic diffusion potential on the uptake of disopyramide, an organic cation, by the brush-border membrane vesicles was different between intestinal and renal cortex preparations [3]. Moreover, the binding of the cationic form of enoxacin, which has a pH-dependent K^+/H^+-diffusion-potential-stimulated transport, was found to play an important role in the permeation process of this drug across the intestinal membrane [4]. However, it remains unclear whether such a stimulation effect of transmembrane potential on the uptake will contribute to the common transport mechanism of many organic cations in intestinal brush-border membrane.

In the present study, the membrane potential dependence of the uptake of a series of organic monovalent cations with different lipophilicity by rat intestinal brush-border membranes was investigated in order to gain further details about the intestinal transport mechanism.

Materials and Methods

Chemicals

Tyramine hydrochloride, tryptamine hydrochloride were obtained from Nacalai Tesque (Kyoto, Japan). L- or D-Tyrosine and D-tryptophan were purchased from Kyowa Hakko Kogyo (Tokyo, Japan). 5-Benzyloxytryptamine (BOTA), 5-benzyloxytryptophan (BOTP) and valinomycin were from Sigma (St. Louis, MO,

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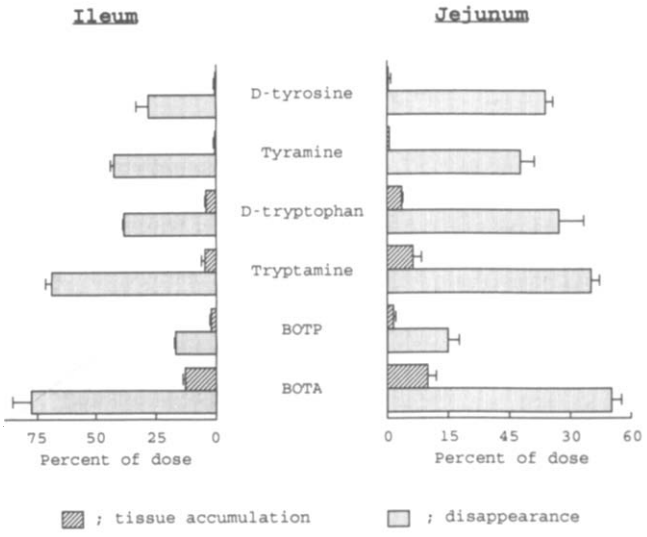

Fig. 1. Absorption behavior of organic cations and their zwitterionic derivatives from rat intestinal loops (jejunum and ileum). Each column represents the mean with S.E. of three or four determinations. Drug concentration was either 0.4 mM (BOTA, BOTP) or 2.0 mM (tryptamine, D-tryptophan, tyramine, D-tyrosine).

USA). L-[^{14}C]tyrosine (13.10 GBq/mmol) was purchased from Amersham (Amersham, UK). All other chemicals were of the highest grade available and used without further purification.

Preparation of membrane vesicles

Brush-border membrane vesicles were isolated from rat small intestine by the $CaCl_2$ precipitation method of Kessler et al. [5] as described previously [6]. Membrane vesicles were suspended in the buffer used for the transport studies. The composition of the utilized buffers are given in the legends.

Transport experiments

The uptake of substrates was measured by a rapid filtration technique as described previously [1,2]. The trapped substrate on the Millipore filter (HAWP, 0.45 μm pore size, 25 mm diameter) was extracted with 300 μl of the mobile phase used for the HPLC analysis.

Absorption study using rat intestinal loops

The absorption experiment was carried out at pH 7.0 using the in situ loop technique of Levine and Pelikan [7]. Either jejunal or ileal loop (10 cm) was prepared in a male Wistar rat (200–250 g). After washing the inside of loop gently with 10 ml of modified Ringer's solution [8], 1 ml of each drug used in this study (BOTA, BOTP, tyramine, D-tyrosine, tryptamine and D-tryptophan), dissolved in modified Ringer's solution, was injected into each loop. After 15 min, the contents of the loop were recovered as completely as possible, and the lumen was washed with modified Ringer's solution to give a total volume of 5 ml. For the determination of tissue concentration of BOTA and BOTP, scraped mucosa was homogenized with saline and diluted to a volume of 5 ml. 1 ml of these samples, loop contents and homogenized mucosa were diluted with 2 ml 100 mM phosphate buffer (pH 2.5) and shaken with 2 ml of chloroform to clean the samples. The aqueous layer was for HPLC injection.

Analytical procedures

The tested compounds in intestinal loop contents, intestinal mucosa and brush-border membrane vesicles were analyzed by HPLC (Hitachi L-6000, Hitachi, Tokyo, Japan) equipped with an F-1000 Fluorometric monitor (Hitachi, Tokyo, Japan). The detailed condi-

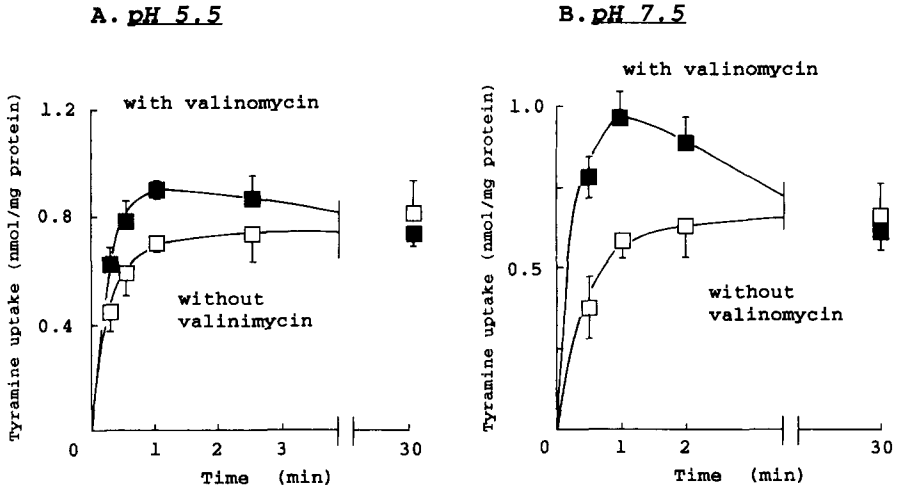

Fig. 2. Effect of a valinomycin-induced K^+-diffusion potential (interior negative) on the uptake of tyramine by rat intestinal brush-border membrane vesicles at pH 5.5. Membrane vesicles were suspended in 100 mM potassium gluconate, 100 mM D-mannitol and either 20 mM Mes-Tris (pH 5.5, panel A) or 20 mM Hepes-Tris (pH 7.5, panel B) buffer in the presence (■) or absence (□) of valinomycin (7 μg/mg protein). The vesicles (40 μl) were incubated with 200 μl of either 20 mM Mes-Tris (pH 5.5, panel A) or 20 mM Hepes-Tris (pH 7.5, panel B) containing 100 mM sodium gluconate, 100 mM D-mannitol and 1.2 mM tyramine. Each point represents the mean with S.E. of six determinations.

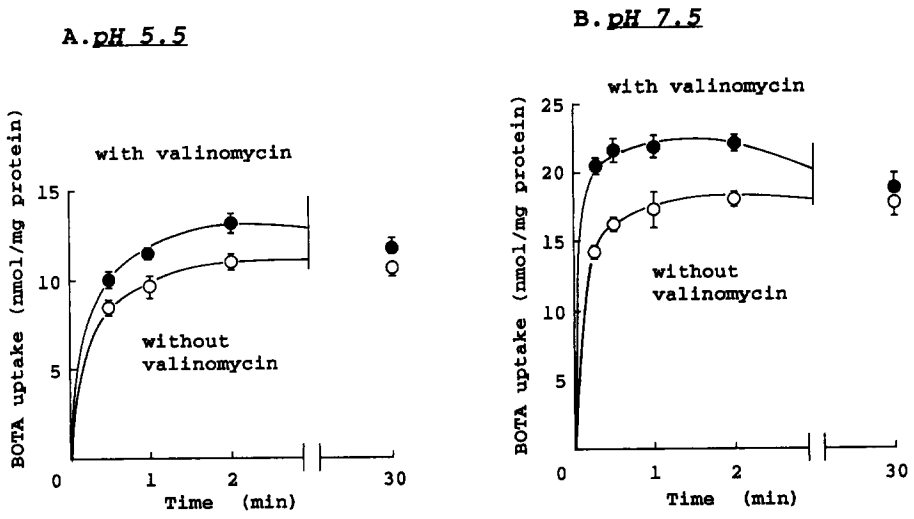

Fig. 3. Increased effect of a valinomycin-induced K^+-diffusion potential (interior negative) on the uptake of BOTA at pH 5.5 and 7.5. The experimental conditions of incubation media were identical to those in Fig. 2, except for substrate concentration (BOTA, 0.3 mM). Symbols: ○, in the absence of valinomycin; ●, in the presence of valinomycin (7 μg/mg protein).

tions of the HPLC such as wavelength, flow-rate, stationary phase and composition of the mobile phase were the same as those in the previous report [1]. The radioactivity of [^{14}C]tyrosine was assayed by a standard liquid scintillation technique. Protein concentrations were determined by the method of Lowry et al. [9] using bovine serum albumin as a standard.

Results

Absorption behavior of organic cations and their zwitterionic derivatives from rat intestinal loop

Fig. 1 shows the absorption results following injection of organic cations (BOTA, tryptamine, tyramine) and their zwitterionic derivatives (BOTP, tryptophan, tyrosine) into either jejunal or ileal loop. To avoid the influence of carrier-mediated transport on the absorption of L-tryptophan and L-tyrosine, D-forms of these amino acids were used for this experiment.

The net absorption of these cations from the lumen was superior to that of their zwitterionic derivatives, except for the tyramine absorption from the jejunal loop. Similarly, the tissue concentrations of these organic cations were also higher than their zwitterions' accumulation.

Stimulation effect of the K^+-diffusion potential (inside-negative) on the uptake of organic cations

In the previous paper, the initial uptake of tryptamine, an organic cation, by the brush-border membrane vesicles was increased in proportion to the degree of ionic diffusion potential across the mem-

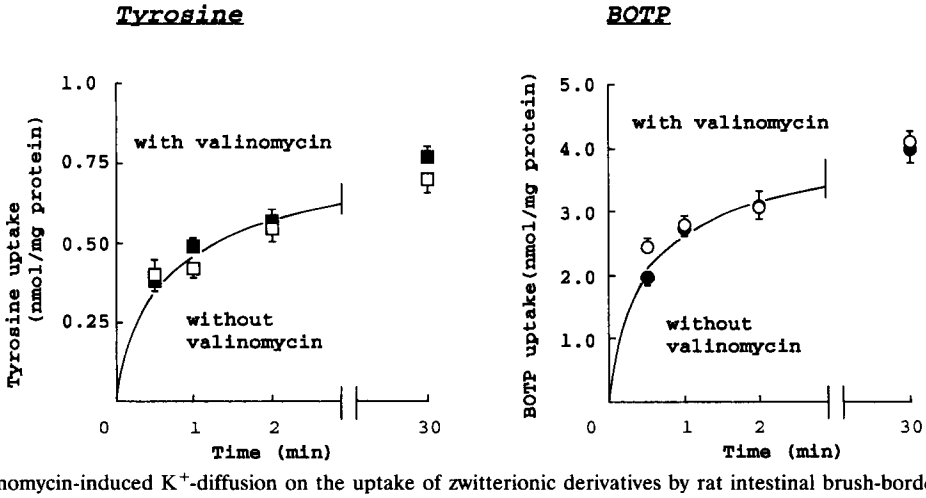

Fig. 4. Effect of a valinomycin-induced K^+-diffusion on the uptake of zwitterionic derivatives by rat intestinal brush-border membrane vesicles. The experimental buffers were same as those in Fig. 2B. Drug concentration were 0.25 mM for BOTP (●, with valinomycin; ○, without valinomycin) and 1.0 mM for tyrosine (■, with valinomycin; □, without valinomycin). Each point represents the mean with S.E. of three or four determinations.

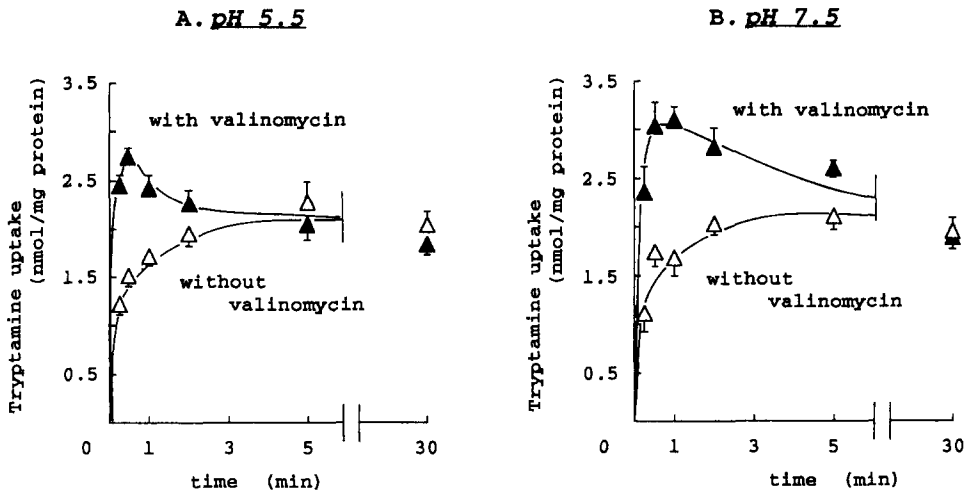

Fig. 5. Higher stimulation effect of K^+-diffusion potential generated by valinomycin (inside negative) on the tryptamine uptake by the brush-border membrane vesicle at pH 7.5 compared with that at pH 5.5 (1.0 mM of tryptamine). Symbols: ▲, in the presence of valinomycin; △, in the absence of valinomycin (7 μg/mg protein). Each point represents the mean with S.E. of six determinations.

brane (inside-negative). To clarify the similarity of the uptake characteristics of other organic cations to that of tryptamine, the effects of K^+-diffusion potential (inside-negative) on the uptake of tyramine and BOTA were examined (Figs. 2 and 3). The initial uptakes of both compounds were also increased by an inside-negative membrane potential, and this result was in good agreement with that of the tryptamine uptake study [2].

On the contrary, no stimulation effect of K^+-diffusion potential was exhibited in the uptake of the zwitterionic compounds such as tyrosine and BOTP even at pH 7.5 (Fig. 4).

The uptake profiles of tryptamine in the presence of K^+-diffusion potential at pH 5.5 and pH 7.5 are depicted in Fig. 5. The stimulation effect of K^+-diffusion potential on the tryptamine uptake was also dependent on the medium pH (pH 7.5 > 5.5). Furthermore, as shown in Figs. 2B, 3B and 5B, there was more distinct stimulation on the initial uptake of these organic cations at pH 7.5. Additionally, the higher uptake values at equilibrium in the medium of pH 7.5 were also observed for BOTA.

Effect of an outward H^+-gradient on the uptake of organic cations by voltage-clamped brush-border membrane vesicles

To elucidate the effect of an outward-directed H^+-gradient on the uptake of organic cations in the membrane vesicles, the tyramine and BOTA uptakes with/without a H^+-gradient were compared. The results are depicted in Figs. 6A and 7A. The initial uptakes of

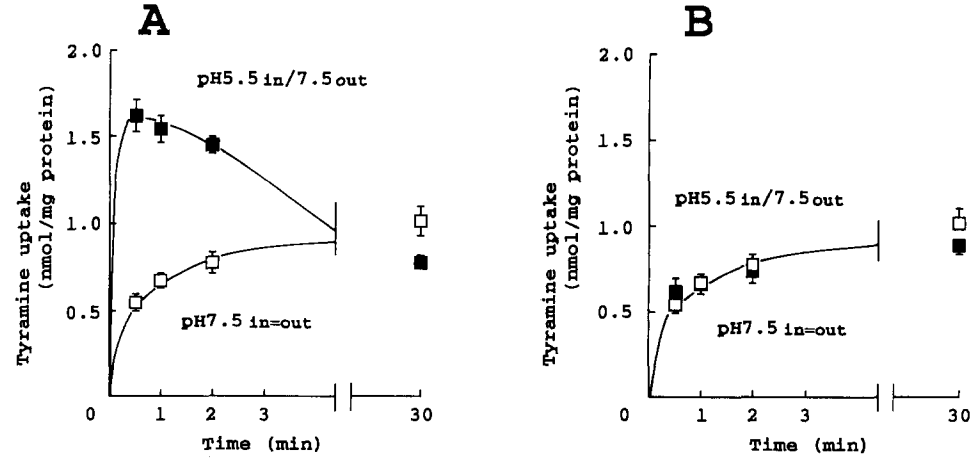

Fig. 6. Tyramine uptake in either non-treated brush-border membrane vesicles (A) or voltage-clamped vesicles (B) in the presence of an outwardly directed H^+-gradient. Membrane vesicles were preloaded with 100 mM D-mannitol plus 100 mM potassium gluconate buffered with either 20 mM Mes-Tris, pH 5.5 (■) or 20 mM Hepes-Tris, pH 7.5 (□). Uptake buffer was 100 mM potassium gluconate plus 100 mM D-mannitol buffered with 20 mM Hepes-Tris (pH 7.5). Tyramine uptake was measured in the absence (A) or presence (B) of valinomycin (7 μg/mg protein). Concentration of tyramine was 1.0 mM. Each point represents the mean with S.E. of six or seven determinations.

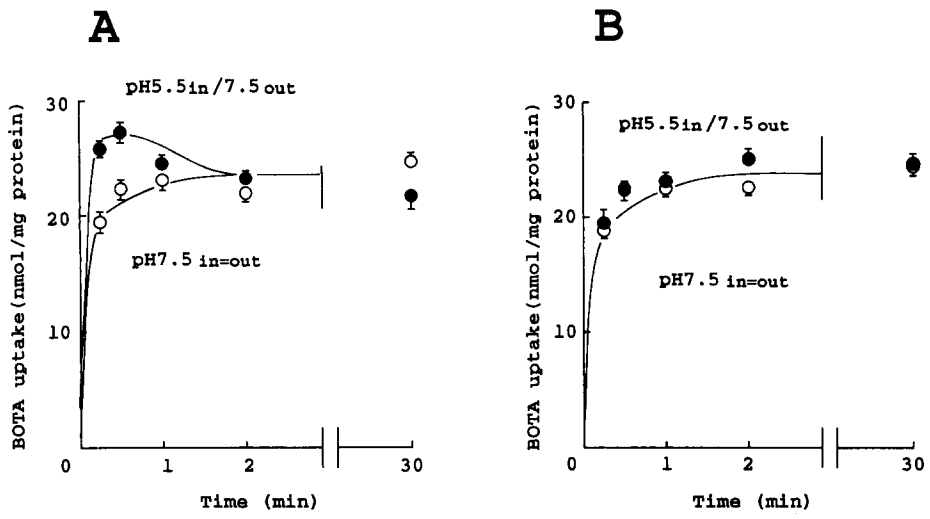

Fig. 7. Uptake of BOTA in either non-treated brush-border membrane vesicle (A) or voltage-clamped vesicles (B) in the presence of an outward H^+-gradient. Membrane vesicles were preloaded with 100 mM D-mannitol plus 100 mM potassium gluconate buffered with either 20 mM Mes-Tris (pH 5.5, •) or 20 mM Hepes-Tris (pH 7.5, ○). Uptake buffer was 100 mM potassium gluconate plus 100 mM D-mannitol buffered with 20 mM Hepes-Tris (pH 7.5). BOTA concentration was 0.25 mM. Each point represents the mean with S.E. of six determinations.

these cations were rapid in the presence of an outward H^+-gradient (pH 5.5_{in} > pH 7.5_{out}) and exhibited transient 'overshoot' phenomena. The H^+-gradient-stimulated uptake was not due to the influence of the H^+-gradient on the intravesicular space, since the equilibrium value, which depends on the intravesicular volume, remained the same in the presence or in the absence of the H^+-gradient.

On the other hand, we also studied the uptake of these cations by voltage-clamped brush-border membrane vesicles in the presence of an outward-directed H^+-gradient. Under this experimental condition, only the H^+-diffusion potential was effectively dissipated while H^+-gradient itself remained [10]. As shown in Figs. 6B and 7B, the voltage-clamped membrane vesicles exhibited a complete disappearance of the overshoot phenomenon. In contrast, there was neither stimulation effect of an outward H^+-gradient nor of the H^+-diffusion potential on the uptake of their zwitterions, tyrosine and BOTP (not shown).

Discussion

In general, the transport process of ionic compounds across the intestinal epithelial cell membrane is the rate-limiting step for their intestinal absorption because these compounds are hydrophilic and ionized at the intestinal pH values. The results obtained in the rat intestinal loop experiment proved that disappearance and tissue accumulation of organic cations (BOTA, tryptamine and tyramine) were greater than of their zwitterionic derivatives. This result seems to be due to the high binding of organic cations to brush-border membrane as mentioned previously [1]. Moreover, the absorption rank order from loops and the binding order to the brush-border membrane vesicles of the cationic compounds were the same. In addition, we have previously demonstrated that the degree of enoxacin uptake as cationic form (pH 5.5) was higher than that as zwitterionic form (pH 7.5) by rat intestinal brush-border membrane vesicles [4]. These findings suggest that the transport of organic cations or cationic forms itself was more rapid than that of their zwitterionic forms in like manner as the binding behavior [1].

In order to determine the net uptake of each compound, we had an attempt to distinguish between the transport into an intravesicular space and the binding to the membrane. The binding value to the membrane was determined by extrapolating the uptake at an infinite extravesicular osmolarity (zero intravesicular space), therefore, subtracting the binding value from the total uptake will result in the net uptake.

However, the resulted net uptakes of the cationic compounds (tyramine, tryptamine, BOTA) were not equal. The intravesicular space calculated to be 0.7–1.1 μl/mg protein on the basis of the equilibrium uptake of D-glucose, can usually accommodate about 0.7–1.1 nmol/mg protein at equilibrium in the presence of 1 mM of D-glucose. Also, in our previous study about enoxacin uptake, we have found from the net uptake of this drug that the vesicle volume was approx. 1.0 μl/mg protein, which was determined by subtracting the binding value (extrapolated to infinite extravesicular osmolarity) from the total uptake [4]. As a result, the differences in the net uptakes of the cationic compound tested in this study are relative to the differences in the transport characterisctics of each compound. A similar result had been observed in the transport study of polyamines (spermine, spermidine, and putrescine) [12,13] and the uptake characteristics of tryptamine

and BOTA seem to be similar to those of polyamines such as spermine and spermidine and putrescine, although more detailed investigation are necessary.

It is appropriate to consider that the pH dependence of the stimulation (Figs. 2 and 3) is caused by the changes of membrane surface charges since the tested organic cations were almost protonated. Bitonti et al. [11] have reported that the uptake of the bis-(benzyl)-polyamine analogue into erythrocytes depended upon the pH of medium. We have also found that the distinct pH dependence was observed in the initial uptake of polyamines such as spermine, spermidine and putrescine, which were completely ionized in the physiological pH, by the intestinal brush-border membrane vesicles [12,13]. Therefore, the present results indicate that transmembrane-transport of cationic compounds can be tightly related to the interaction with membrane surface charge.

The study of cationic compounds uptake by voltage-clamped brush-border membrane vesicles have exhibited a complete disappearance of overshoot phenomenon even in the presence of an outward H^+-gradient. A recent study using rabbit intestinal brush-border membrane vesicles has shown that the uptake of guanidine into these vesicles participated in H^+ coupled antiport systems [10]. In the renal and liver plasma membrane, many investigators [14–17] have reported that the organic cations such as N_1-methylnicotinamide and cimetidine were transported via H^+-antiport systems. These organic-H^+-antiport systems are insensitive to changes in membrane potential, and are linked to an outwardly directed H^+-gradient as the driving force for organic cation uptake [10,14–16]. Nevertheless, we have found out that disopyramide uptake related to a interior negative H^+-diffusion potential into the intestinal brush-border membrane vesicles apparently seems to be similar to the overshoot uptake of this drug via H^+-antiport system in the renal brush-border membrane [3]. As a conclusion, in the intestinal membrane, these result provide strong evidence for the presence of a common permeation mechanism of organic cations, except for a few compounds such as guanidine [15]. This permeation mechanism is depending upon the transmembrane potential, but not participated in H^+-antiport system. Furthermore, it is considered that this membrane potential-dependent transport mechanism can be linked with the binding of organic cation into the brush-border membrane at the initial uptake stage.

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