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Effect of membrane surface potential on the uptake and the inhibition of cationic compounds in rat intestinal brush-border membrane vesicles and liposomes

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Abstract

The effect of membrane surface potential on the uptake of tryptamine, an organic cation, by rat intestinal brush-border membrane vesicles was investigated. In the presence of an inside-negative K^+-diffusion potential, the manner of initial uptake of tryptamine appeared to be pH-dependent and the uptake in the acidic medium was lower than that in the neutral medium. Changes in surface potential of brush-border membrane vesicles were monitored using 8-anilino-1-naphthalenesulfonic acid (ANS) and the results suggested that the membrane surface potential (negative charge on the membrane surface) decreased in the acidic medium. A good correlation was observed between the K^+-diffusion potential-dependent uptake of tryptamine and membrane surface potential monitored by ANS at various pH levels. The uptake of tryptamine by liposomes (large unilamellar vesicles), which contained various amounts of dipalmitoylphosphatidylserine (DPPS), was also examined. The uptake of tryptamine decreased with a decrease of DPPS content in the liposomes, and was correlated with the membrane surface potential monitored by ANS. Moreover, the effect of organic cations on the uptake of tryptamine by intestinal brush-border membrane vesicles was examined. The uptake of tryptamine was inhibited by tetracaine and imipramine. The inhibitory effect of these cations was well correlated with changes in the membrane surface potential in the presence of tetracaine or imipramine. These results suggest that the K^+-diffusion potential-dependent uptake of tryptamine by intestinal brush-border membrane vesicles is affected by membrane surface potential, and the inhibition of tryptamine uptake originates in changes in the membrane surface potential caused by the organic cations.

Keywords: Organic cation; Tryptamine; Surface potential; Liposome; Inhibition; Brush-border membrane ; (Rat intestine)

1. Introduction

There have been many investigations concerning the transport mechanisms of organic cations using brush-border membrane vesicles. It has been reported that tetraethylammonium [1–4], N^1-methylnicotinamide [5–8], cimetidine [9,10], guanidine [4], etc. are transported via organic cation-H^+ antiport systems in renal brush-border membrane vesicles. In contrast, there are few reports concerning the carrier-mediated transport systems of organic cations in small intestinal brush-border membrane vesicles, although Miyamoto et al. [11] reported a guanidine-H^+ antiport system in rabbit intestinal brush-border membrane vesicles.

Recently, we have examined the transport mechanism of cationic compounds (tryptamine [12,13], enoxacin [14], disopyramide [15]) using rat intestinal brush-border membrane vesicles. We mentioned that the stimulation of uptake of these cations by an outward H^+-gradient is not due to the antiport system, but due to electrophoretic mobility driven by the H^+-diffusion potential. The diffusion-potential-dependent uptake of these cations inhibited one another, but the details of the mechanism of the inhibition were not ascertained, except in the participation of binding of these cations to the membrane [16,17].

We have reported that the permeation rate of several anionic compounds into rat intestinal brush-border membrane vesicles and liposomes is dependent on surface

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonate; Val, valinomycin; DPPC, dipalmitoylphosphatidylcholine; DPPS, dipalmitoylphosphatidylserine.

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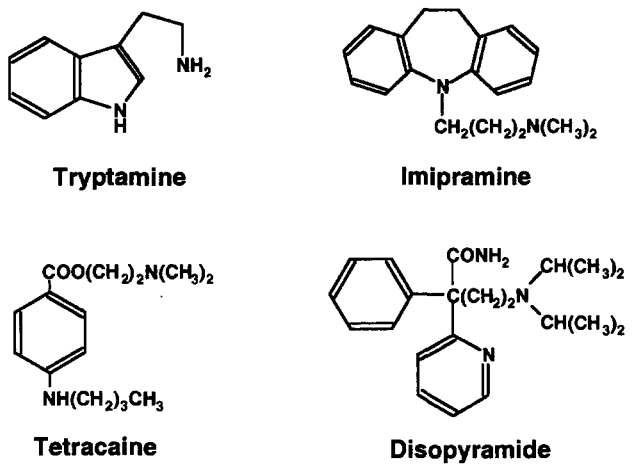

Fig. 1. Structures of tested compounds.

potential originating in a surface negative charge [18,19]. Moreover, Schafer et al. reported that biguanide derivatives change the surface potential of mitochondria and phospholipid liposomes [20,21]. Therefore, it is likely that there is an electrostatic interaction between organic cations and the brush-border membrane, and that its change due to binding of the organic cations to the membrane contributes to the mechanism of uptake and the inhibitory effect.

In this study, we investigated the membrane-surface-potential dependency of the uptake of tryptamine by rat intestinal brush-border membrane vesicles and liposomes (large unilamellar vesicles) in order to elucidate the mechanism of membrane transport and the inhibition of organic cations. The structures of tested organic cations are given in Fig. 1.

2. Materials and methods

2.1. Chemicals

Tryptamine hydrochloride and 8-anilino-1-naphthalenesulfonate magnesium were purchased from Nakalai Tesque (Kyoto, Japan). Tetracaine hydrochloride, imipramine hydrochloride, disopyramide, valinomycin, azolectin, dipalmitoyl-L-α-phosphatidylcholine (DPPC) and dipalmitoyl-DL-α-phosphatidylserine (DPPS) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of the highest grade available and used without further purification.

2.2. Preparation of intestinal brush-border membrane vesicles

Brush-border membrane vesicles were isolated from rat whole intestine by $CaCl_2$ precipitation [22] as described previously [23]. Membrane vesicles were suspended in the 20 mM Mes-Tris buffer containing 100 mM D-mannitol and 100 mM potassium gluconate.

2.3. Preparation of liposomes (large unilamellar vesicles)

Liposomes were prepared by the reversed phase evaporation technique [24] as described previously [18] with some minor modifications. The lipid mixture (azolectin (equivalent to 8 μmol phosphorus), DPPC and DPPS (2 μmol in total)) in chloroform were added to a 10 ml round-bottomed flask, and solvent was removed by a rotary evaporator. The lipids were redissolved in 3 ml of diethyl ether and 0.5 ml of a buffer (100 mM D-mannitol, 100 mM potassium gluconate, 20 mM Hepes-Tris (pH 7.5)) was added. The mixture was sonicated in a bath-type sonicator (UT-204, Sharp, Osaka, Japan), under nitrogen for 5 min. The mixture was then placed on a rotary evaporator and the organic solvent was removed under vacuum. Following the vortex mixing, the suspension was evaporated to remove traces of the organic solvent.

2.4. Uptake experiments

The uptake of substrates was measured by a rapid filtration technique as described previously [25]. When the brush-border membrane vesicles were used, the reaction was initiated by addition of 100 μl of a buffer containing the substrate to 20 μl of a membrane vesicles suspension (10–15 mg protein/ml) at 25°C. In the case of the liposomes, the reaction was initiated by addition of 500 μl of substrate solution to 20 μl of a liposomes suspension (about 20 μmol phosphorus/ml) at 25°C. At a predetermined time, the reaction was stopped by diluting the reaction mixture with 5 ml of ice-cold buffer (150 mM NaCl, 20 mM Hepes-Tris buffer (pH 7.5)). The tube contents were immediately filtered through a Millipore filter (HAWP, 0.45 μm, 2.5 cm diameter) and were washed once with 8 ml of the same ice-cold buffer. The substrate trapped on the filter was extracted with 1 ml of a mixture containing 0.25 M acetic acid and 25% dimethylsulfoxide, and was measured by HPLC.

2.5. Analytical method

The concentrations of tryptamine were determined by HPLC (Hitachi L-6000) equipped with an 820-FP spectrofluorometer (JASCO, Tokyo, Japan) at an excitation wavelength of 285 nm and an emission wavelength of 350 nm. Separation was achieved on a reversed phase column (ODS, Hitachi 3053, dp = 5 μm, 4 mm i.d., 250 mm) using a mobile phase consisting of acetonitrile/0.05 M KH_2PO_4 (1:3). Protein concentrations were determined by the method of Lowry et al. [26] with bovine serum albumin as the standard. Phospholipid was determined by the method of Bartlett [27].

2.6. Measurement of surface potential change of membrane vesicles

Changes in the surface potential of brush-border membrane vesicles and liposomes were monitored by measuring the changes in the fluorescence intensity of ANS, which has been widely used to measure the surface potential of the membranes [20,28–30] as described previously [18] with some modifications. The measurements were carried out in a spectrofluorometer (650-60, Hitachi, Tokyo, Japan) with an excitation wavelength of 385 nm and emission wavelength of 480 nm. The temperature was maintained at 25°C. To 1 ml of the vesicle suspension, 1 ml of dye solution (0, 12.5, 25, 50, 100 μM) was added. Final concentrations of vesicles were 0.1 mg protein/ml for brush-border membrane vesicles, 0.75 μmol phospholipid/ml for liposomes. Following this, the fluorescence intensity was measured. Corrections for the background fluorescence and light scattering were made with blanks containing brush-border membrane vesicles alone or dye alone. Fluorescence intensity, f, is defined as

$$f = f_a - (f_d + f_m) \quad (1)$$

where f_a, f_d and f_m are the fluorescence intensity of a membrane vesicle-ANS suspension, ANS solution alone and a membrane vesicle suspension alone, respectively. From the ordinate intercept of double reciprocal plots of fluorescence intensity against ANS concentration, maximum fluorescence intensity, F, was calculated. Relative membrane surface potential, Ψ_{rel}, was calculated by the following equation,

$$\Psi_{rel} = F_s/F_c \quad (2)$$

where F_s and F_c stand for the maximum fluorescence intensity of treated and control vesicles.

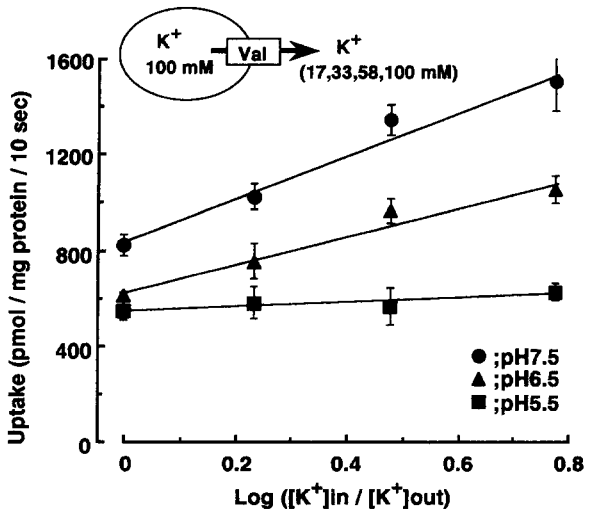

Fig. 2. Correlation between K^+-diffusion potential and initial uptake of tryptamine by brush-border membrane vesicles at various pH values. Vesicles (20 μl) were incubated with 100 μl of either 20 mM Mes-Tris (pH 5.5–pH 6.5) or 20 mM Hepes-Tris (pH 7.5) buffer containing 0.6 mM tryptamine, 100 mM D-mannitol and various concentrations of potassium gluconate (0, 20, 50, 100 mM, and various concentrations of sodium gluconate to maintain the osmolarity). Final concentration of valinomycin was 7 μg/mg protein. Each point represents the mean ± S.E. of 3–9 measurements.

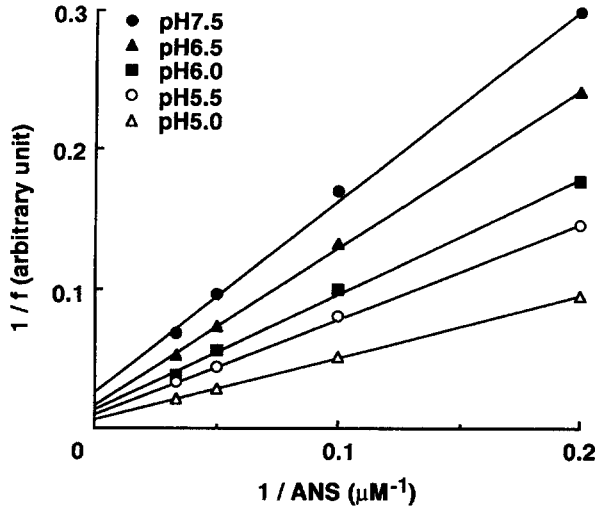

Fig. 3. Double reciprocal plot of ANS and fluorescence intensity of ANS. Effect of pH of medium on the surface potential of brush-border membrane vesicles (regression coefficients: ●; 0.999, ▲; 1.000, ■; 0.999, ○; 1.000, △; 1.000).

3. Results

3.1. Effect of the pH of the medium on the K^+-diffusion potential-dependent uptake of tryptamine by intestinal brush-border membrane vesicles

Fig. 2 shows the initial uptake of tryptamine by intestinal brush-border membrane vesicles in the presence of a K^+-diffusion potential (inside-negative) at pH 5.5, 6.5 and 7.5. The uptake of tryptamine was dependent on the K^+-diffusion potential and was well correlated with $\log([K^+]_{in}/[K^+]_{out})$ in all cases. However, the slope, i.e., the diffusion potential-dependent uptake of each condition, was different, and it was lower in the acidic medium than in the neutral medium.

3.2. Relation between the uptake of tryptamine and membrane surface potential

Fig. 3 shows a double reciprocal plot of ANS concentration and fluorescence intensity of ANS which reflects the surface potential of membrane vesicles. The ordinate intercept decreased in the acidic medium, which reflects a decrease of surface charge negativity. The relationship between the pH of the medium and Ψ_{rel} calculated by Eq. (2) for a control of vesicles at pH 7.5 is shown in Fig. 4. As shown in Fig. 5, a good correlation was observed

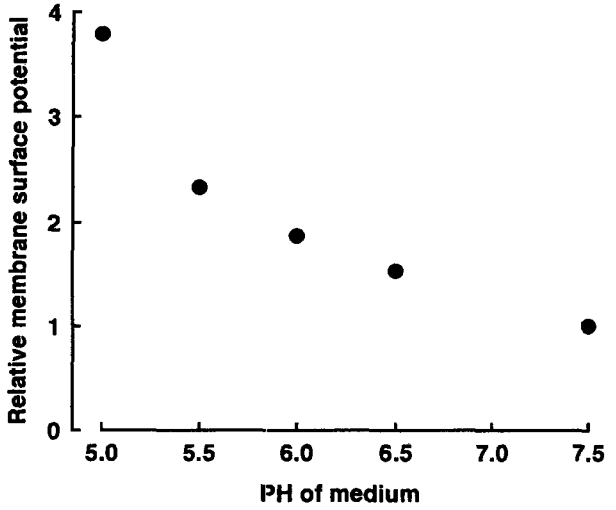

Fig. 4. Relation between the pH of medium and relative membrane surface potential (Ψ_{rel}) of brush-border membrane vesicles.

between Ψ_{rel} and the K^+-diffusion potential-dependent uptake of tryptamine (slopes in Fig. 2) suggesting a participation of surface potential in the uptake of tryptamine by intestinal brush-border membrane vesicles.

3.3. Effect of membrane surface potential on the uptake of tryptamine by liposomes

Fig. 6 shows the relation between the DPPS content of liposomes and Ψ_{rel} at a control of 0% DPPS liposomes. It is obvious that the surface potential (negative surface charge) of liposomes increased with an increase of the DPPS content. The uptake of tryptamine by liposomes containing 0, 2, 5% DPPS in the presence of a K^+-diffusion potential (inside-negative) was measured (Fig. 7). The

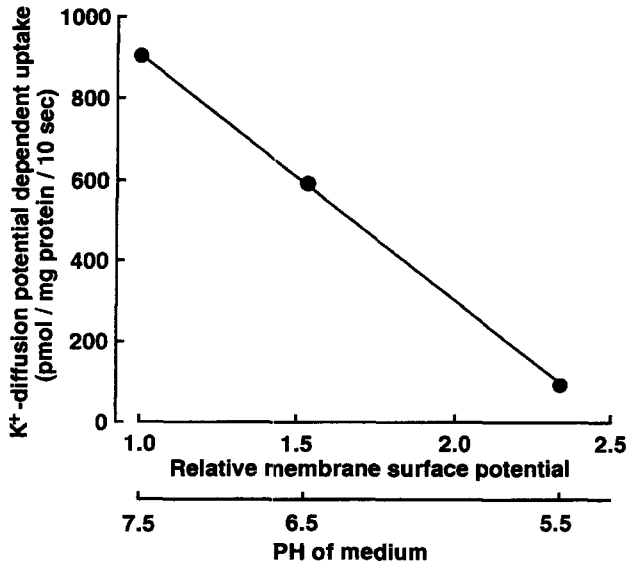

Fig. 5. Correlation between relative membrane surface potential and K^+-diffusion potential dependent uptake of tryptamine at various pH values.

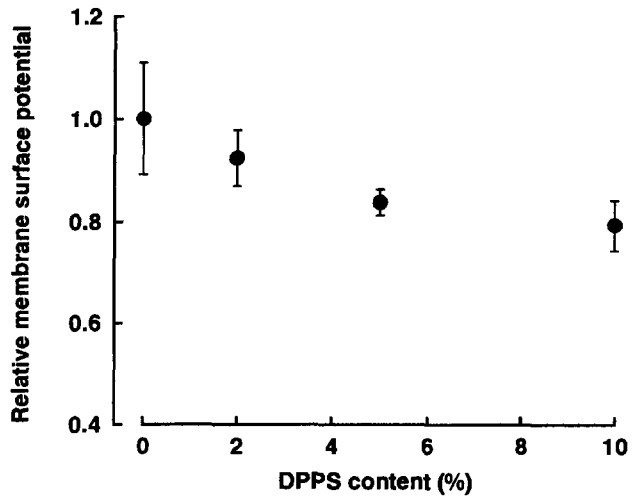

Fig. 6. Changes in the membrane surface potential as a function of DPPS content in liposomes. Each point represents the mean ± S.E. of three measurements.

initial (0.5 and 5 min) uptake of tryptamine increased with an increase of DPPS, and beside which, a good correlation was observed between the uptake of tryptamine and Ψ_{rel}. These results were consistent with the results obtained from the study using brush-border membrane vesicles.

3.4. Effect of ionic strength on the uptake of tryptamine by intestinal brush-border membrane vesicles

To clarify the dependency of tryptamine uptake on membrane surface potential, we examined further the ef-

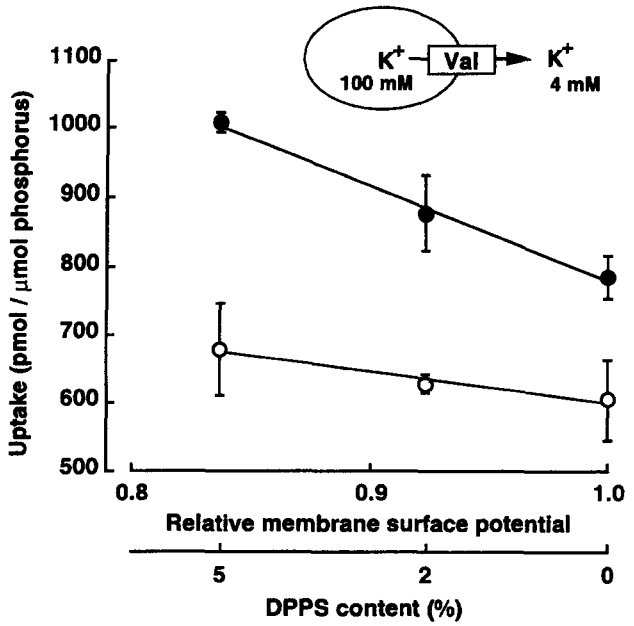

Fig. 7. Correlation between membrane surface potential and initial uptake of tryptamine (○; 30 s, ●; 5 min) by liposomes containing various amounts of DPPS in the presence of K^+-diffusion potentials (inside-negative). Final concentration of valinomycin was 1 μg/μmol phosphorus. Each value represents the mean ± S.E. of three measurements.

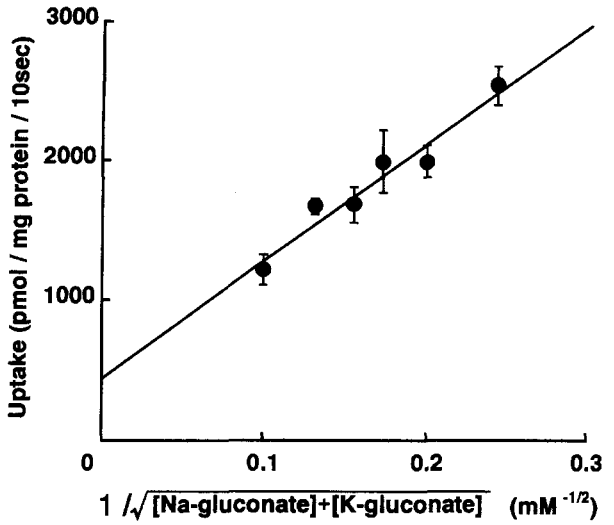

Fig. 8. Effect of ionic strength of the external medium on the uptake of tryptamine by rat intestinal brush-border membrane vesicles. Vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris (pH 7.5) buffer containing 100 mM D-mannitol and various concentrations of sodium gluconate (0, 10, 20, 30, 50, 100 mM, and various concentrations of D-mannitol to maintain the osmolarity). Final concentration of external potassium gluconate was 16.7 mM. Each point represents the mean ± S.E. of three measurements.

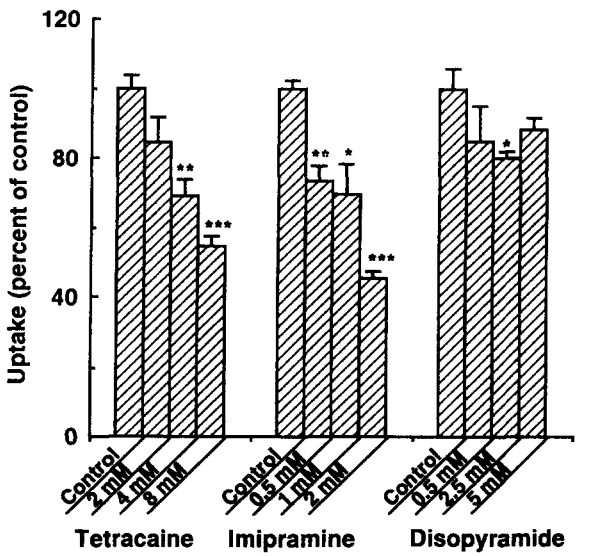

Fig. 9. Effect of organic cations on the initial (10 s) uptake of tryptamine. Vesicles (20 μl) suspended in 20 mM Hepes-Tris (pH 7.5) buffer containing 100 mM D-mannitol and 100 mM potassium gluconate were incubated with 100 μl of 20 mM Hepes-Tris (pH 7.5) buffer containing 100 mM D-mannitol, 100 mM sodium gluconate and various concentrations of organic cations. Final concentration of external potassium gluconate was 16.7 mM. Each value represents the mean ± S.E. of 3–6 measurements. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different from control.

fect of ionic strength of the external medium on the uptake of tryptamine by rat intestinal brush-border membrane vesicles. In terms of the Gouy-Chapman model, in the case of 1:1 electrolytes, the surface charge density (σ) is predicted to be related to the surface potential (ψ_0) by:

$$\sigma = (8C\epsilon kT)^{1/2} \sinh(e\psi_0/2kT) \quad (3)$$

where k and T have their usual meaning, C, ϵ and e are the bulk concentration of the salt, dielectric constant and elementary charge, respectively. At low potential, Eq. (3) can be approximated to:

$$\sigma = (2e^2\epsilon C/kT)^{1/2}\psi_0 \quad (4)$$

namely, surface potential (ψ_0) is inversely proportional to the square root of the salt concentration.

Fig. 8 shows the effect of the ionic strength of the external medium on the uptake of tryptamine by rat intestinal brush-border membrane vesicles. The uptake of tryptamine was increased with decrease of the concentration of sodium gluconate, and was proportional to the reciprocal of the square root of the salt concentration.

3.5. Effect of organic cations on the uptake of tryptamine and surface potential of brush-border membrane vesicles

The effect of tetracaine, imipramine and disopyramide on the initial uptake of tryptamine by brush-border membrane vesicles in the presence of a K^+-diffusion potential (inside-negative) was studied. As shown in Fig. 9, the uptake of tryptamine was inhibited by tetracaine and imipramine. The inhibitory effect was concentration dependent, and the effect of imipramine was stronger than tetracaine. On the other hand, little inhibition was observed in the presence of disopyramide. The effect of these organic amines on the surface potential of brush-border membrane vesicles is shown in Fig. 10. Changes in the surface potential were observed in the presence of tetracaine and imipramine, but disopyramide had no effect. These effects correspond with the degree of inhibition of tryptamine uptake by these amines. As shown in Fig. 11, the uptake of tryptamine was well correlated with the

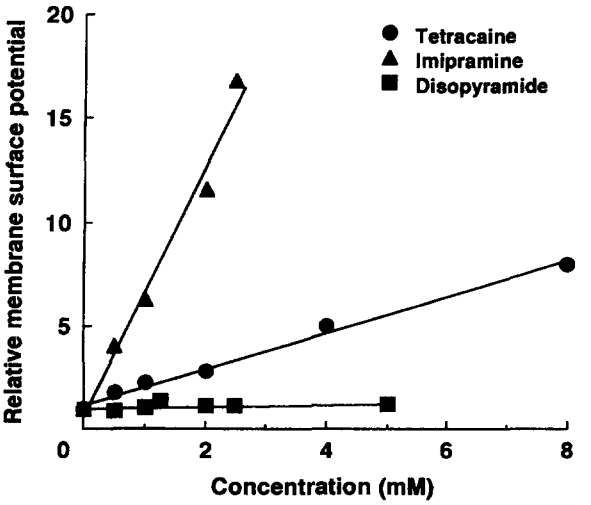

Fig. 10. Effect of organic cations on the surface potential of brush-border membrane vesicles.

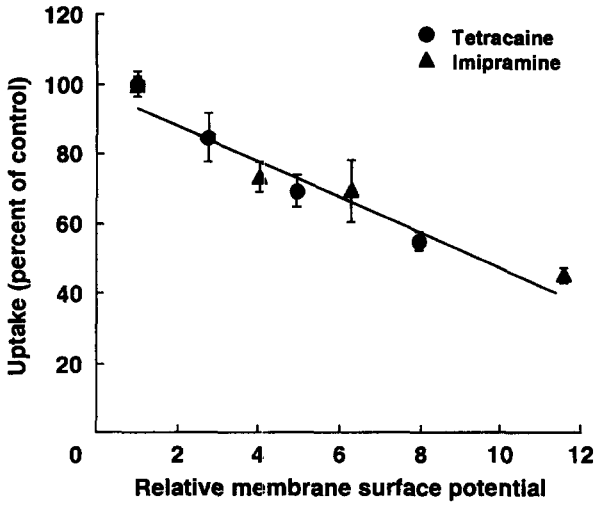

Fig. 11. Correlation between relative membrane surface potential and initial (10 s) uptake of tryptamine by brush-border membrane vesicles in the presence of tetracaine or imipramine. Vesicles (20 μl) suspended in 20 mM Hepes-Tris (pH 7.5) buffer containing 100 mM D-mannitol and 100 mM potassium gluconate were incubated with 100 μl of 20 mM Hepes-Tris (pH 7.5) buffer containing 100 mM D-mannitol, 100 mM sodium gluconate and various concentrations of ether tetracaine or imipramine. Final concentration of external potassium gluconate was 16.7 mM. Each point represents the mean ± S.E. of 3–6 measurements.

relative membrane surface potential in the presence of tetracaine or imipramine. Moreover, the effect was common to both of these amines.

4. Discussion

Previously, we have reported that the uptake of some organic cations, including tryptamine, into intestinal brush-border membrane vesicles is stimulated by an inside-negative H^+- or K^+-diffusion potential and the rate of this electrophoretic uptake is affected by changes in the pH of the medium or the addition of organic cations [12,13]. In this study, we investigated the mechanism of the inhibitory effect on the uptake of tryptamine, an organic cation, from the viewpoint of the change in the membrane surface potential.

As shown in Fig. 2, the K^+-diffusion potential dependent uptake of tryptamine by intestinal brush-border membrane vesicles decreased with a decrease in the pH of the medium. Since tryptamine has a high pK_a value (10.3) [31], it is almost completely ionized for the tested pH values of the medium (5.5–7.5). We, therefore, took note of the state of the membrane surface. A good correlation was observed between the surface potential of brush-border membrane vesicles monitored by ANS and the K^+-diffusion potential dependent uptake of tryptamine for each pH of the medium (Fig. 5). It is possible, however, that the unionized form of tryptamine, of which there exists only a small amount but which is affected by changes in the pH of the medium, contributes to the uptake. Therefore, we studied the uptake of tryptamine by liposomes (large unilamellar vesicles) to clarify the relation between the uptake of tryptamine and membrane surface potential. This method is useful to study the effect of membrane surface potential because it can be regulated by the addition of DPPS without any change in the pH of the medium. The uptake of tryptamine by liposomes in the presence of a K^+-diffusion potential increased with an increase of the DPPS content and was well correlated with the surface potential of liposomes (Fig. 7). In addition, the uptake of tryptamine by rat intestinal brush-border membrane vesicles was increased with decrease of ionic strength of the external medium (Fig. 8). These results suggest that the membrane surface potential contributes to the transport of tryptamine across the intestinal brush-border membrane. It is considered that the electrostatic interaction between tryptamine and the brush-border membrane is lowered by a decrease of the surface negative charge.

The mechanism of the inhibition by organic cations on the uptake of tryptamine by intestinal brush-border membrane vesicles was also investigated from the viewpoint of membrane surface potential. It has been reported that imipramine and tetracaine bind to biological membranes [16,32,33]. However, there are few reports concerning the relationship between the changes in the membrane surface potential and the inhibition of membrane transport. As shown in Fig. 9, if considered from the standpoint of concentration dependency, imipramine had stronger inhibitory effect than tetracaine. On the other hand, the plot of the relationship between the uptake of tryptamine and the relative membrane surface potential in the presence of tetracaine or imipramine shows that it was more or less constant (Fig. 11).

These results suggest that, in addition to the diffusion potential (driving force), the electrostatic interaction between organic cations and the brush-border membrane is an important factor in the transport of organic cations. It is also suggested that the mutual inhibition of the transport of organic cations through the intestinal brush-border membrane is caused by the change in surface potential due to the binding of these cations to the membrane.

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