Biochimica et Biophysica Acta, 1111 (1992) 145–150

BBAMEM 75777

Membrane-potential-dependent uptake of tryptamine by rat intestinal brush-border membrane vesicles

Mitsuru Sugawara, Makoto Sasaki, Ken Iseki and Katsumi Miyazaki

Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Sapporo (Japan)

(Received 13 May 1992)

Key words: Tryptamine; Brush-border membrane; Membrane potential; Membrane transport

The effect of membrane potential on the uptake of tryptamine, an organic cation, by rat intestinal brush-border membrane vesicles was studied. In the presence of an outwardly directed H^+-gradient, the initial uptake of tryptamine was stimulated remarkably and the overshoot phenomenon was observed. In contrast, the uptake was depressed by an inwardly-directed H^+-gradient. The effect of H^+-gradient on the uptake of tryptamine was maintained in the presence of FCCP, whereas it vanished when voltage-clamped vesicles were used. Moreover, the uptake of tryptamine was linearly augmented with increase of the valinomycin-induced inside-negative K^+ diffusion potential. These results suggest that tryptamine is taken up into intestinal brush-border membrane vesicles depends upon the ionic diffusion potential. The effect of several indole derivatives and amine compounds on the uptake of tryptamine was also examined. The uptake of tryptamine was inhibited by all amine compounds used, but anionic and zwitterionic compounds had no effect, suggesting that these amines interact on brush-border membrane and cause an inhibitory effect.

Introduction

The transport mechanisms of cationic compounds have been investigated repeatedly on brush-border membrane vesicles, mainly prepared from rat and rabbit kidney. As the results of these studies, it has been reported that organic cations (tetraethylammonium [1–4], *N*-methylnicotinamide [5–8], cimetidine [9,10], etc.) are transported via organic cation-H^+ antiport mechanism(s) on renal brush-border membrane. On the other hand, studies concerning the carrier-mediated transport of organic cations in the small intestinal brush-border membrane are few.

Recently, we examined the transport mechanisms of an organic cation, disopyramide, using rat intestinal and renal brush-border membrane vesicles, and uncovered evidence that the stimulation of disopyramide uptake by an outward H^+-gradient was due to disopyramide-H^+ antiport in the renal brush-border membrane and caused by a H^+ diffusion potential (inside negative) in the intestinal brush-border membrane [11]. Moreover, as for the uptake of enoxacin, the stimulation by inside negative diffusion potential was observed in the cationic form in rat intestinal brush-border membrane vesicles [12].

In this study, membrane-potential dependency and effects of other cations on the uptake of tryptamine, a model compound of organic cation, by rat intestinal brush-border membrane vesicles were investigated.

Materials and Methods

Chemicals. Tryptamine hydrochloride was purchased from Nakalai Tesque (Kyoto, Japan). L-Tryptophan was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). Gramine and indole acetic acid were purchased from Wako (Osaka, Japan). Chlorpromazine hydrochloride, imipramine hydrochloride, desipramine hydrochloride, valinomycin and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) were purchased from Sigma (St. Louis, MO, USA). 3,3'-Dipropylthiadicarbocyanine iodide (diS-C_3(5)) was obtained from Molecular Probes (Junction City, Eugene, OR, USA). All other chemicals were of the highest grade available and used without further purification.

Preparation of intestinal brush-border membrane vesicles. Brush-border membrane vesicles were isolated

Correspondence to: K. Miyazaki, Department of Pharmacy, Hokkaido University Hospital, School of Medicine Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

Abbreviations: Mes, 2-(*N*-morpholino)ethanesulfonic acid; Hepes, *N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; diS-C_3(5), 3,3'-dipropylthiadicarbocyanine iodide; CPZ, chlorpromazine; IPM, imipramine; DPM, desipramine.

from rat whole intestine by $CaCl_2$ precipitation [13] as described previously [14]. Membrane vesicles were suspended in the buffer used for the transport studies. The composition of the buffer is given in the figure legends.

Uptake experiments. The uptake of substrates was measured by a rapid filtration technique as described previously [15]. The reaction was initiated by addition of 100 μl of a buffer containing the substrate to 20 μl of membrane vesicle suspension (10–15 mg of protein/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) which was washed once with 8 ml of the same ice-cold buffer. The substrate trapped on the filter was extracted with 300 μl of mobile phase for HPLC (described below) and was measured by HPLC.

Analytical methods. Tryptophan and tryptamine were determined by HPLC (Hitachi L-6000, Hitachi Tokyo, Japan) equipped with an F-1050 fluorescence spectrophotometer (Hitachi, 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, 5 μ, 4 mm i.d., 250 mm) using a mobile phase consisting of acetonitrile: 0.05 M phosphate buffer (1:4; (pH 2.5)). Protein concentrations were determined by the method of Lowry et al. [16] with bovine serum albumin as standard.

Measurement of membrane-potential changes. Changes in the membrane-potential were monitored by measuring the changes in the fluorescence intensity of diS-C_3(5) according to the methods of Ganapathy et al. [17], which was applied to rabbit intestinal brush-border membrane vesicles, with some modifications. The measurements were carried out in a spectrofluorometer (650-60, Hitachi, Tokyo, Japan) with an excitation wavelength of 622 nm and emission wavelength of 670 nm. The temperature was maintained at 25°C. Membrane vesicles were suspended in the buffer (0.1 mg protein/ml) and a stock dye solution (1 mM in ethanol) was added. The final concentration of the dye was 1 μM. The transient change of fluorescence was measured in the presence of K^+- or H^+-gradient. The condition of each experiment and the composition of the buffer are given in the figure legends.

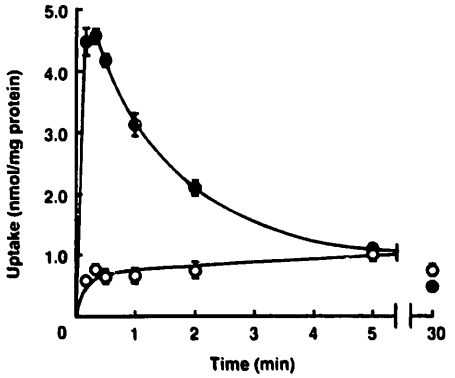

Fig. 2. Time-course of uptake of tryptamine in the presence or absence of outwardly directed H^+-gradient. Membrane vesicles were preincubated in 100 mM KCl, 100 mM D-mannitol and either 20 mM Mes-Tris buffer (pH 5.5) (●) or 20 mM Hepes-Tris buffer (pH 7.5) (○) THe vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris buffer (pH 7.5), containing 0.6 mM tryptamine, 100 mM D-mannitol and 100 mM KCl. Each point represents the mean ± S.E. of three measurements.

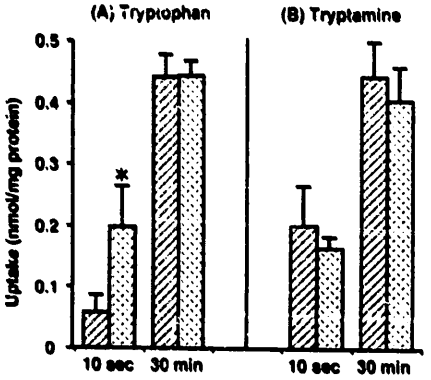

Fig. 1. Effect of Na^+-gradient on the uptake of tryptophan (A) and tryptamine (B) by rat intestinal brush-border membrane vesicles. Membrane vesicles were preincubated in 100 mM KCl, 100 mM D-mannitol and 20 mM Hepes-Tris buffer (pH 7.5). The vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris buffer (pH 7.5), containing 0.6 mM substrate, 100 mM D-mannitol and either 100 mM KCl (■) or 100 mM NaCl (▨). Each value represents the mean ± S.E. of three measurements. * $P < 0.05$ significantly different from control.

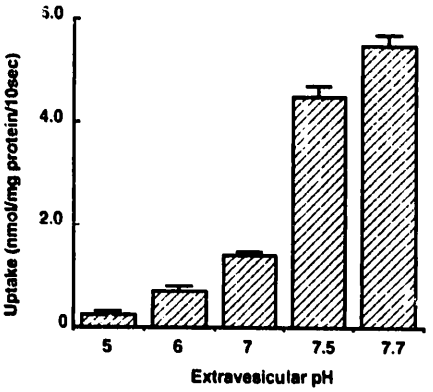

Fig. 3. Effect of various pH conditions of extravesicler medium on the initial uptake of tryptamine. The vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 20 mM Mes-Tris buffer (pH 5.5). The vesicles (20 μl) were incubated with 100 μl of either 20 mM Mes-Tris buffer (pH 5, 6) or 20 mM Hepes-Tris buffer (pH 7, 7.5, 7.7), containing 0.6 mM tryptamine, 100 mM D-mannitol and 100 mM KCl. Each value represents the mean ± S.E. of three measurements.

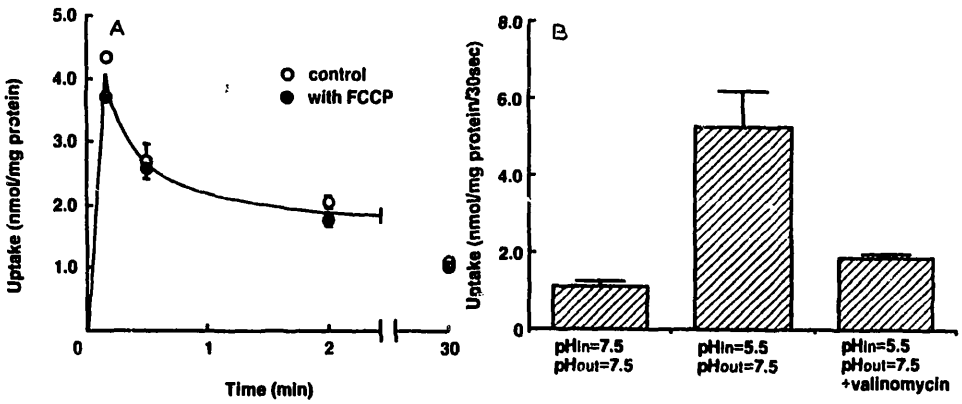

Fig. 4. Effect of FCCP (A) and valinomycin (B) on the uptake of tryptamine in the presence of outward H^+ gradient. (A) Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 20 mM Mes-Tris buffer (pH 5.5). The vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris buffer (pH 7.5), containing 0.6 mM tryptamine, 100 mM D-mannitol and 100 mM KCl in the presence (●) or absence (○) of 50 μM FCCP. (B) Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and either 20 mM Mes-Tris buffer (pH 5.5) or 20 mM Hepes-Tris buffer (pH 7.5) in the presence or absence of valinomycin (7 μg/mg protein). The vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris buffer (pH 7.5), containing 1.2 mM tryptamine, 100 mM D-mannitol and 100 mM KCl.

Results

Effect of Na^+- and H^+-gradients on the uptake of tryptamine

To compare the transport of tryptamine with that of amino acid, L-tryptophan, the effect of Na^+- and H^+-gradients on their uptake by brush-border membrane vesicles was studied. The initial uptake of L-tryptophan was stimulated remarkably in the presence of an inwardly directed Na^+-gradient, but the uptake of tryptamine was not affected (Fig. 1). On the other hand, in the presence of an outwardly directed H^+ gradient (pH_{in} 5.5, pH_{out} 7.5), only the uptake of tryptamine was stimulated and notable overshoot phenomenon was observed (Fig. 2). Moreover, stimulation of tryptamine uptake was dependent on the degree of H^+ gradient as shown in Fig. 3. These data suggest that tryptamine is not transported via the transport system for amino acid (tryptophan).

Effect of ionophore on the uptake of tryptamine

Fig. 4A shows the time-course of uptake of tryptamine with or without FCCP in the presence of an outward H^+-gradient (pH_{in} 5.5, pH_{out} 7.5). Momentary disappearance of H^+-gradient by FCCP did not affect the uptake of tryptamine. Fig. 4B shows the uptake of tryptamine by voltage-clamped vesicles. In this experiment, K^+ was presented in equimolar concentrations both inside and outside the vesicles and valinomycin was added to the vesicle suspension beforehand. Therefore, ionic diffusion potential is immediately compensated by K^+ movement. In this condition, the tryptamine uptake was not increased even in the presence of an outward H^+ gradient. The effect of inward H^+-gradient was also examined. The initial uptake of tryptamine was decreased in the presence of an inward H^+-gradient ($pH_{in} = 7.5$, $pH_{out} = 5.5$). This effect was not observed when voltage-clamped vesicles were used (Fig. 5).

Changes in the membrane-potential by H^+ diffusion

In order to confirm the fluorescence change of diS-C_3(5) reflects the membrane-potential change of brush-border membrane, the fluorescence change caused by K^+ diffusion potential was measured. The fluorescence is linearly changed with logarithmic concentration of K^+ in an external medium. This result suggest that the fluorescence change well reflects the membrane-potential change (Fig. 6A). The fluorescence change caused by H^+ gradient was also measured. H^+ gradient specific fluorescence change was determined by excluding the non-specific fluorescence intensity obtained from voltage clamped vesicles. As shown in Fig. 6B, the fluorescence is linearly changed

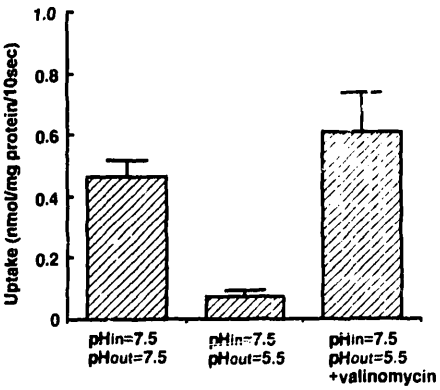

Fig. 5. Effect of valinomycin on the uptake of tryptamine in the presence of inward H^+-gradient. Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and Hepes-Tris buffer (pH 7.5) in the presence or absence of valinomycin (7 μg/mg protein). The vesicles (20 μl) were incubated with 100 μl of either 20 mM Mes-Tris buffer (pH 5.5) or 20 mM Hepes-Tris buffer (pH 7.5), containing 0.6 mM tryptamine, 100 mM D-mannitol and 100 mM KCl.

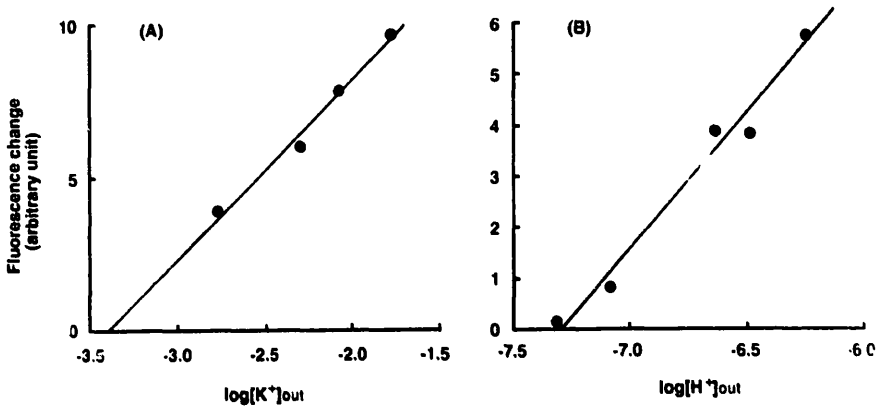

Fig. 6. Changes in the fluorescence intensity of diS-C_3(5) as a function of K^+ (A) and H^+ (B) diffusion potential in rat intestinal brush-border membrane vesicles. (A) The vesicles were preincubated in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM Hepes-Tris buffer (pH 7.5), containing valinomycin (7 μg/mg protein). A constant volume (200 μl) of 20 mM Hepes-Tris buffer (pH 7.5), containing 100 mM D-mannitol and various concentrations of K-gluconate (add Na-gluconate to maintain the osmolarity) was added to 1 ml of vesicle suspension. (B) The vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 1 mM Hepes-Tris buffer (pH 7.5). To 1 ml of vesicle suspension, 200 μl of 20 mM Hepes-Tris or Mes-Tris buffer (different pH) containing 100 mM D-mannitol and 100 mM KCl. The maximal transient fluorescence increase was corrected for non-transient fluorescence changes due to dilution.

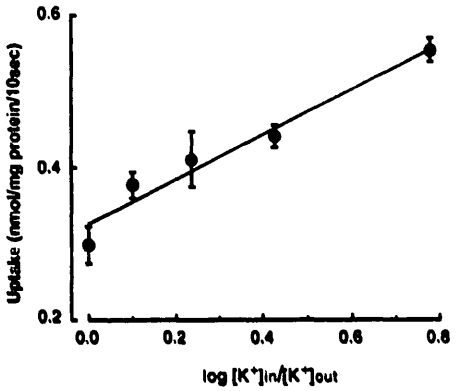

Fig. 7. Correlation between the uptake of tryptamine and valinomycin-induced inside-negative K^+ diffusion potential. Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM K-gluconate and 20 mM Hepes-Tris buffer (pH 7.5). The vesicles (20 μl) were incubated with 100 μl of 20 mM Hepes-Tris buffer (pH 7.5), containing 100 mM D-mannitol and various concentrations of K-gluconate (0–100 mM, add Na-gluconate to maintain the osmolarity). Each point represents the mean ± S.E. of three measurements.

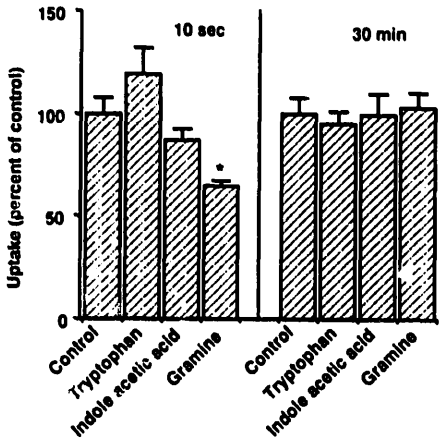

Fig. 8. Effect of indole derivatives on the uptake of tryptamine. The experimental conditions are as given in Fig. 2. Final concentration of each compound was 5 mM. Each value represents the mean ± S.E. of three measurements. * $P < 0.05$ significantly different from control.

with log $[H^+]$ of outer medium, suggesting the generation of a H^+ diffusion potential.

Tryptamine uptake depends on the K^+ diffusion potential

The uptake of tryptamine in the presence of various K^+ diffusion potential is shown in Fig. 7. The initial uptake of tryptamine was increased by an inside-negative membrane potential and was well correlated with the logarithm of the ratio of intravesiclar $[K^+]$ to extravesiclar $[K^+]$.

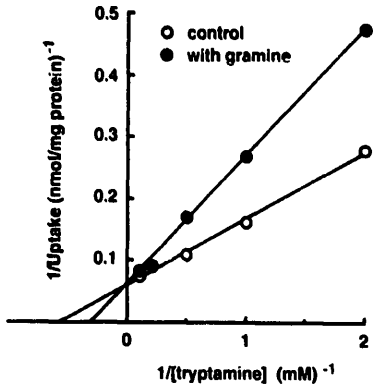

Fig. 9. Lineweaver-Burk plots of the initial uptake (10 s) of tryptamine in the presence (●) or absence (○) of gramine (5 mM). The experimental conditions are as given in Fig. 2. The concentration of tryptamine was 0.5–10 mM.

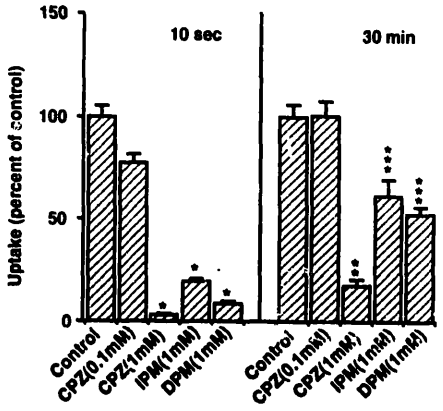

Fig. 10. Effect of organic amines on the uptake of tryptamine. The experimental conditions are as given in Fig. 2. Each value represents the mean ± S.E. of three measurements. * $P < 0.005$, ** $P < 0.01$, *** $P < 0.05$, significantly different from control.

Effect of indole derivatives and organic amines on the uptake of tryptamine

In order to clarify the structural similarities in the membrane potential dependent uptake, the effects of indole derivatives and organic amines on the uptake of tryptamine were examined. Uniquely, only the cationic compound, gramine, inhibited the uptake of tryptamine when gramine (cation), tryptophan (zwitterion) or indole acetic acid (anion) was introduced (Fig. 8). As shown in Fig. 9, the uptake of tryptamine appeared to be saturable with an apparent K_m of 1.85 mM and V_{max} of 17.09 nmol/mg protein/10 s. Moreover, the Lineweaver-Burk plots of the tryptamine uptake (10 s) in the presence of gramine exhibited a competitive type of inhibition with K_i of 3.18 mM. It was also demonstrated that, in the gramine uptake, a clear overshoot phenomenon was observed in the presence of an outwardly directed H^+-gradient (not shown). Fig. 10 shows the effects of several amphiphilic cationic drugs on the uptake of tryptamine. Chlorpromazine, imipramine and desipramine remarkably inhibited the tryptamine uptake, at an even lower concentration than gramine.

Discussion

In this study, it was suggested that tryptamine is taken up into intestinal brush-border membrane vesicles dependent upon ionic diffusion potential and not due to the exchange with H^+. The observations which support this conclusion are as follows: (1) The uptake of tryptamine was stimulated by an outwardly directed H^+ gradient and decreased by an inwardly directed H^+ gradient. This effect was dependent on the degree of H^+ gradient (Fig. 2). (2) The change of the tryptamine uptake caused by H^+ gradient was not affected by FCCP addition and the effect of a H^+ gradient was not observed when voltage-clamped vesicles were used (Figs. 4A, 4B and 5). (3) The fluorescence change of diS-C_3(5) suggested that the diffusion potential was generated by H^+-gradient (Fig. 6B). (4) The uptake of tryptamine was linearly increased with log $[K^+]_{in}/[K^+]_{out}$ (Fig. 7).

There have been reports stating that the uptake of organic cations are stimulated by an outwardly-directed H^+-gradient using renal brush-border membrane vesicles and the organic cation-H^+ antiport system was proposed as a mechanism for this transport [1–10]. On the other hand, little is known concerning their transport system withir the intestinal brush-border membrane. Recently, Miyamoto et al. reported a guanidine-H^+ antiport system in rabbit intestinal brush-border membrane [18]. In the study utilizing rat renal and intestinal brush-border membrane vesicles, we obtained evidence that disopyramide, a cationic drug, is transported by the H^+-antiport system in renal brush-border membrane, but in intestinal brush-border membrane, stimulation of its uptake by an outward H^+-gradient is caused by an inside-negative H^+ diffusion potential [11]. Furthermore, the uptake of the cationic form of enoxacin by rat intestinal brush-border membrane vesicles increased remarkably in the presence of H^+ and K^+ generated an inside negative membrane-potential [12]. However, correlation between uptake and membrane-potential change or measurement of H^+ diffusion potential have never been examined. In this study, in order to confirm what is the driving force for tryptamine uptake, the effect of ionophore on the uptake of tryptamine was studied. In the presence of FCCP, it is expected that the uptake of tryptamine is enhanced by larger membrane potential induced by rapid H^+ diffusion if membrane potential is involved in providing the driving force for tryptamine uptake. On the other hand, it has been reported that the uphill transport via H^+-organic cation antiport system was inhibited and that the overshoot phenomenon disappeared in the presence of protonophore [8,10,11]. As shown in Fig. 4A, FCCP did not affect the uptake of tryptamine even in the presence of an outward H^+ gradient. Moreover, the both effects of outward and inward H^+ gradient disappeared in the voltage-clamped membrane vesicles (Figs. 4B, 5). Moreover, changes of membrane potential by H^+ diffusion were monitored with a potential-sensitive dye (diS-C_3(5)) (Fig. 6B) and there was a good correlation between the initial uptake of tryptamine and log $[K^+]_{in}/[K^+]$out (Fig. 7). Although it is unclear why the tryptamine uptake was not increased by FCCP in the presence of outward H^+-gradient, both our previous and our present results suggest that cationic compounds uptake into intestinal brush-border membrane vesicles is mainly dependent on the membrane-potential.

In this study, the effect of several indole derivatives and organic amines on the uptake of tryptamine were

also examined in the presence of an outwardly directed H^+ gradient. All of the cationic compounds used in this study inhibited the uptake of tryptamine and yet tryptophan and indole acetic acid exhibited no effects at all. From the Lineweaver-Burk plots, it was suggested that the inhibition of tryptamine uptake by gramine was a competitive type. In addition, the uptake of gramine was stimulated in the presence of an outward H^+ gradient (not shown). Moreover, the remarkable inhibitory effects of amphiphilic drugs (chlorpromazine, imipramine and desipramine) were observed and the 30-min uptake was also inhibited. It is reported that these drugs considerably bind to intestinal brush-border membrane [19] and that their uptake is facilitated by transmembrane electrical potential differences following after the binding phase in the early time [20]. It can be considered that the membrane disruption did not occur at these conditions, because we have already reported that chlorpromazine (1 mM) did not affect the equilibrium uptake (30 min) of zwitterionic β-lactam antibiotics [21]. Although these inhibitory effects may be due to the binding to the membrane, it is impossible to determine the binding in distinction from the intravesiclar component of uptake amounts at this stage utilizing membrane vesicles.

In conclusion, it was suggested that some of the organic cations are transported driven by membrane potential differences in the intestinal brush-border membrane. As for this mechanism, the binding of organic cations to the brush-border membrane may be an important process.

References

1 Takano, M., Inui, K., Okano, T., Saito, H. and Hori, R. (1984) Biochim. Biophys. Acta 773, 113–124.

2 Inui, K., Saito, H. and Hori, R. (1985) Biochem. J. 227, 199–203.

3 Rafizadeh, C., Roch-Ramel, F. and Schali, C. (1986) J. Pharm. Exp. Ther. 240, 308–313.

4 Wright, S.H. and Wunz, T.M. (1987) Am. J. Physiol. 253, F1040–1050.

5 Holohan, P.D. and Ross, C.R. (1981) J. Pharm. Exp. Ther. 216, 294–298.

6 Sokol, P.P., Holohan, P.D. and Ross, C.R. (1985) J. Pharm. Exp. Ther. 233, 694–699.

7 Wright, S.H. (1985) Am. J. Physiol. 249, F903–F911.

8 Sokol, P.P., Holohan, P.D., Grass, S.M. and Ross, C.R. (1988) Biochim. Biophys. Acta 940, 209–218.

9 Takano, M., Inui, K., Okano, T. and Hori, R. (1985) Life Sci. 37, 1579–1585.

10 McKinney, T.D. and Kunnemann, M.E. (1987) Am. J. Physiol. 252, F525–F535.

11 Takahashi, Y., Itoh, T., Kobayashi, M., Sugawara, M., Saitoh, H., Iseki, K., Miyazaki, K., Miyazaki, S., Takada, M. and Kawashima, Y. (1992) J. Pharm. Pharmacol. in press.

12 Iseki, K., Hirano, T., Fukushi, Y., Kitamura, Y., Miyazaki, S., Takada, M., Sugawara, M., Saitoh, H. and Miyazaki, K. (1992) J. Pharm. Parmacol. in press.

13 Kessler, M., Acuto, O., Strelli, H., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154.

14 Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K. and Arita, T. (1989) J. Pharm. Pharmacol. 41, 628–632.

15 Sugawara, M., Saitoh, H., Iseki, K., Miyazaki, K. and Arita, T. (1990) J. Pharm. Pharmacol. 42, 314–318.

16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.

17 Ganapathy, V., Burckhardt, G. and Leibach, F.H. (1985) Biochim. Biophys. Acta 816, 234–240.

18 Miyamoto, Y., Ganapathy, V. and Leibach F.H. (1988) Am. J. Physiol. 255, G85–G92.

19 Saitoh, H., Kawai, S., Iseki, K., Miyazaki, K. and Arita, T. (1988) J. Pharm. Pharmacol. 40, 776–780.

20 Saitoh, H., Kawai, S., Iseki, K., Miyazaki, K. and Arita, T. (1989) J. Pharm. Pharmacol. 41, 200–202.

21 Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K. and Arita, T. (1988) J. Pharm. Pharmacol. 40, 701–705.