Ionic strength has a greater effect than does transmembrane electric potential difference on permeation of tryptamine and indoleacetic acid across Caco-2 cells

Mitsuru Sugawara [a], Megumi Kurosawa [a], Kasumi Sakai [a], Michiya Kobayashi [a], Ken Iseki [b], Katsumi Miyazaki [a],*

[a]*Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060-8648, Japan*
[b]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*

Abstract

The effects of transmembrane electric potential difference and ionic strength on the permeation of tryptamine and indoleacetic acid across a Caco-2 cell monolayer were examined. A decrease in the transmembrane electric potential difference caused by the addition of potassium ion to the transport buffer had no effect on the permeation rate of either compound. On the other hand, an increase in ionic strength resulted in a decrease in the permeation rate of tryptamine and an increase in the permeation rate of indoleacetic acid. The changes in the permeation rate with changes in the ionic strength were correlated with the membrane surface potential monitored by 1-anilino-8-naphthalenesulfonic acid (ANS), a fluorescent probe. We tested these effects using several other cationic and anionic compounds. These effects of ionic strength were found to be common to all drugs tested. The compound that showed a relatively lower permeation rate was given relatively stronger effect. The possibility of overestimation or underestimation caused by these effects should be considered when the permeation of an ionic compound is evaluated using a cell monolayer system. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

Electrostatic interaction between ionic drugs and a biological or artificial membrane affect the permeation rate of the drugs across the membrane. We previously reported that the rate of uptake of tryptamine, a cationic compound, into rat intestinal brush-border membrane (BBM) vesicles was stimulated in the presence of inside-negative transmembrane electric potential difference [1]. This electrophoretic permeation was also observed when an artificial membrane (liposomes) was used [2]. Moreover, this phenomenon was common to many cationic compounds [3,4]. The permeation of these cationic compounds was also affected by membrane surface charge and ionic strength. The permeation rate increased when the membrane surface negative charge of liposomes was increased by adding dipalmitoyl-DL-α-phosphatidylserine to the lipid bilayer and also when the ionic strength of a medium of suspended BBM vesicles was decreased [2]. On the other hand, the permeation rates of anionic compounds such as benzylpenicillin, cefixime, and benzyloxyindoleacetic acid decreased with an increase in membrane surface negative charge, whereas transmembrane electric potential difference had no effect [5–7].

Intestinal epithelial cells have negatively charged BBMs and transmembrane electric potential difference (~ -60 mV). Therefore, changes in ionic strength and transmembrane electric potential difference could affect the permeation rates of these cationic and anionic compounds through the epithelial cell layer. Caco-2 cells, a human colon adenocarcinoma cell line, have been used as a model to study the intestinal absorption of drugs because they form a monolayer of enterocyte-like cells that have functional properties of the epithelium [8]. This cell line has been shown to be a good model for studying intestinal drug absorption [9]. In this study, we used this cell line to study the permeation rates of drugs through a monolayer of epithelial cells.

* Corresponding author. Tel.: +81-11-706-5680; fax: +81-11-756-1505.
E-mail address: katsumim@med.hokudai.ac.jp (K. Miyazaki).

The aim of this study is to clarify the degree to which the abovementioned transmembrane electric potential difference-dependent and ionic strength-dependent mechanisms participate in the permeation of drugs through the intestinal epithelium, because it is still not clear whether these mechanisms are actually work in the permeation through epithelial cells (transcellular transport). The concentration of potassium ions in the transport buffer was changed to determine the effects of transmembrane electric potential difference and ionic strength on the permeation of drugs. Changes in the concentration of potassium ions would cause changes in the transmembrane electric potential difference.

2. Materials and methods

2.1. Materials

[^{3}H]Glycylsarcosine (92.8 GBq/mmol) was purchased from Moravek Biochemicals Inc. (Brea, CA). Atenolol, benzyloxytryptamine hydrochloride, imipramine hydrochloride, metoprolol tartrate, pindolol, promethazine hydrochloride, ritodrine hydrochloride, enoxacin, 5-benzyloxy-indole-3-acetic acid, naproxen, tolmetin sodium dihydrate, and fluorescein isothiocyanate–dextran 4000 (FITC–dextran 4000) were purchased from Sigma Chemical Co. (St. Louis, MO). Chlorpromazine hydrochloride, gramine, pheniramine maleate, acetanilide, antipyrine, caffeine, 3-indoleacetic acid, and salicylic acid were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 4-Phenyl-butylamine was obtained from Aldrich Chemical Co. (Milwaukee, WI). 1-Anilino-8-naphthalenesulfonic acid (ANS) hemi-magnesium salt and tryptamine hydrochloride were obtained from Nacalai Tesque (Kyoto, Japan). Metiazic acid was a gift from Mect Co., Ltd. (Tokyo, Japan). The human colon carcinoma cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD). High-glucose Dulbecco's modified Eagle's medium (DMEM),

fetal bovine serum, MEM nonessential amino acids, L-glutamine, and trypsine were purchased from Life Technologies, Inc. (Gaithersburg, MD). All other chemicals were of the highest grade available.

2.2. Cell culture

Caco-2 cells (passage number 32–60) were cultured in DMEM supplemented with 10% fetal bovine serum, 1% MEM nonessential amino acids, and 4 mM L-glutamine. The cells were maintained in 75-cm^2 flasks and trypsinized (0.25% trypsin, 0.02% EDTA in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS)) when they reached confluence. For the experiment to measure the uptake into the cells, 2×10^5 cells were seeded per well in 6-well culture plates (Becton Dickinson, Franklin Lakes, NJ) and then used on the 14th day after seeding. For the measurement of transport through a monolayer of the cells, 6×10^5 cells were seeded on a culture insert (polycarbonate filter; pore size, 3.0 mm; 4.71 cm^2) inside a transwell cell culture chamber (Costar, Cambridge, MA) and used on the 20th day of seeding. For the preparation of BBM vesicles, cells were cultured for 14 days in 10-cm dishes (Becton Dickinson).

2.3. Buffers for uptake and transport experiments

The compositions of the buffers on the apical side used for the uptake and the transport experiments are shown in Table 1. In this study, we reduced the concentrations of the salts in the apical side buffers in order to cause changes in the membrane surface potential by varying ionic strength effectively. The ionic strength was varied by the addition of potassium gluconate. The osmolality and the ionic strength were compensated with D-mannitol and choline chloride, respectively. The pH of the medium was adjusted to 6.0 or 7.4 by 10 mM of either Mes/Tris or Hepes/Tris, respectively. Unless otherwise stated, the pH of the apical buffer was 6.0. The composition of the buffer on the basolateral side was 10

Table 1
Composition of apical buffer

Component	Varied ionic strength				Constant ionic strength			
	2.5 mM K$^+$	25 mM K$^+$	50 mM K$^+$	125 mM K$^+$	2.5 mM K$^+$	25 mM K$^+$	50 mM K$^+$	125 mM K$^+$
CaCl$_2$ (μM)	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
KCl (mM)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
K-gluconate (mM)	0	22.5	47.5	122.5	0	22.5	47.5	122.5
KH$_2$PO$_4$ (μM)	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
MgSO$_4$ (μM)	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
NaCl (mM)	15	15	15	15	15	15	15	15
Choline chloride (mM)	0	0	0	0	122.5	100	75	0
Mes or Hepes (mM)	10	10	10	10	10	10	10	10
D-Mannitol (mM)	257	212	162	11.9	11.9	11.9	11.9	11.9
Na$_2$HPO$_4$ (μM)	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
D-Glucose (mM)	25	25	25	25	25	25	25	25
Ionic strength (I)	17.6	40.1	65.1	140	140	140	140	140

mM Hepes/Tris (pH 7.4), 137 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl$_2$, 0.8 mM MgSO$_4$, 0.44 mM KH$_2$PO$_4$, 0.34 mM Na$_2$HPO$_4$, and 25 mM D-glucose.

2.4. Uptake experiment

The culture medium was removed and the cells were washed twice with 2 ml of uptake buffer (Table 1) and pre-incubated in 2 ml of the buffer at 37 °C for 5 min. Then the uptake was started by replacing the buffer with 2 ml of the buffer containing the drug to be tested. At a pre-determined time, the uptake was terminated by aspiration of the drug solution. The cells were then washed twice with 2 ml of the ice-cold buffer. The cells were solubilized with 1 ml of 1 N NaOH, and 10 μl of an aliquot was used for protein estimation. The rest of the lysate was neutralized with 990 μl of 1 N HCl and the same volume of uptake buffer. After centrifugation at 9000 × g for 5 min, the concentrations of drugs in the supernatant were measured.

2.5. Transport experiment

Cultured Caco-2 monolayers with transepithelial electrical resistance of more than 600 Ω cm^2 were used. The cells were pre-incubated for 5 min at 37 °C in 1.5 ml of apical buffer and 2.6 ml of basal buffer. Then 0.75 ml of the apical side buffer was replaced by the same volume of the drug solution. The culture insert was transferred to the next well containing fresh basal buffer every 15 min for 1 h. The drug concentration of each basal buffer was measured and its flux rate was calculated.

2.6. Preparation of BBM vesicles from Caco-2 cells

Preparation of BBM vesicles from Caco-2 cells was done on ice or at 4°C according to the method of Inui et al. [10], who reported the method to prepare BBM vesicles from a kidney epithelial cell line, LLC-PK1, with some modifications. The cells were scraped and suspended in 40 ml of buffer A (100 mM D-mannitol, 5 mM EGTA, 12 mM Tris–HCl, pH7.1). Then the cells were centrifuged at 200 × g for 10 min and resuspended in 40 ml of buffer A. The cells were disrupted by the nitrogen cavitation method (cell disruption bomb, Parr Instrument Co., Moline, IL). The cells were pressurized at 700 psi (49 kg/cm^2) for 15 min. The collected homogenate was diluted with the same volume of distilled water and homogenized using a Dounce-type homogenizer. Magnesium chloride was added at the final concentration of 0.625 mM, and the mixture was allowed to stand for 15 min and then centrifuged at 3000 × g for 15 min. The supernatant was centrifuged at 27,000 × g for 30 min, and the pellet was resuspended in 40 ml of buffer B (50 mM D-mannitol, 2.5 mM EGTA, 6 mM Tris–HCl, pH 7.1). Then magnesium precipitation and centrifugation as mentioned above were repeated. The final pellet was homogenized using a Dounce-type homogenizer in the same buffers as those used in the uptake and transport experiments. The specific activity of alkaline phosphatase, a marker enzyme of the BBM, of final vesicles was increased by more than sevenfold compared with the initial homogenate. The validity of membrane vesicles was confirmed by the results of an uptake experiment. Sodium gradient-dependent uptake of glutamic acid was observed (not shown).

2.7. Measurement of changes in the surface potential of Caco-2 BBM vesicles

Changes in the surface potential of Caco-2 BBM vesicles were monitored by measuring the changes in the fluorescence intensity of ANS as described previously [2,5]. The measurement was carried out at room temperature in a spectrofluorometer (Hitachi 650-60, Tokyo, Japan) with an excitation wavelength of 385 nm and an emission wavelength of 480 nm. To 300 μl of a suspension of membrane vesicles, 300 μl of dye solution and 600 μl of apical buffer, the ionic strength of which was varied (Table 1), were added. The final concentrations of ANS were 0, 12.5, 50, and 100 μM, and that of BBM vesicles was 0.1 mg protein/ml.

2.8. Distribution coefficient

The *n*-octanol-buffer distribution coefficient at pH 6.0 was measured with buffers having various ionic strengths. The drug solution was shaken with *n*-octanol at room temperature for 30 min. After centrifugation at 1500 × g for 5 min, the concentration of the drug in the aqueous phase was determined.

2.9. Analytical methods

Protein concentration was determined by the method of Lowry et al. [11] using bovine serum albumin as a standard. The concentrations of drugs were determined using an HPLC (Hitachi L-6000) equipped with a Hitachi L-4200H UV detector and Hitachi F-1050 fluorescence detector. Separation of drugs was done on a reversed-phase column Hitachi Gel #3053 except for the case of ritodrine (ERC-ODS-1160, ERC Inc., Tokyo, Japan). The mobile phase was basically 20 or 50 mM KH$_2$PO$_4$ containing various concentrations (15–70 %) of acetonitrile.

3. Results and discussion

3.1. Viability of Caco-2 cells

In this study, we reduced the concentrations of the ions in the apical buffers for the uptake and the transport experiments. Since low concentrations of salts, especially Ca^{2+} and Mg^{2+}, might affect the viability of the monolayer of Caco-2 cells, we measure the transport of FITC–dextran,

which is widely used as a marker for paracellular permeation. The cumulative amount of the transported FITC–dextran to the basal side over a period of 60 min was less than 0.5% of that added to the apical side, and it was almost the same as the value with the normal buffer. On the other hand, when a Ca^{2+}- and Mg^{2+}-free buffer was used, the amount of FITC–dextran increased significantly (not shown). These results suggest that the viability of the monolayer of Caco-2 cells was maintained in the buffer in this study even when the concentrations of divalent cations were reduced.

3.2. Effect of potassium ion concentration on the uptake of glycylsarcosine

It is widely known that Caco-2 cells express PEPT1, an electrogenic proton–peptide symporter [12]. To examine the effect of potassium ions, the transport of [^{3}H]-glycylsarcosine, a substrate of PEPT1, was studied. Fig. 1A shows the effect of potassium ions on the uptake of glycylsarcosine. When sodium was replaced by potassium, the uptake of glycylsarcosine decreased by ~ 50% compared with that in the presence of sodium. As shown in Fig. 1B, the uptake of glycylsarcosine was stimulated in the buffer at pH 6.0. At pH 6.0, the uptake decreased by ~ 40% when potassium in the uptake buffer was increased (from 2.5 to 125 mM). This result suggests that potassium reduces the electrogenic uptake of glycylsarcosine by decreased transmembrane electric potential difference and also that the transport buffers in this study can be used to change the transmembrane electric potential difference.

3.3. Effect of potassium ion concentration on the permeation of tryptamine and indoleacetic acid

Fig. 2 shows the effect of potassium ion concentration on the time course of the permeation of a cationic compound, tryptramine (Fig. 2A,B), and its carboxylic acid derivative, indoleacetic acid (Fig. 2C,D), through a Caco-2 cell mono-

Fig. 2. Effects of potassium concentration of the apical buffer on the permeation of tryptamine (A, B) and indoleacetic acid (C, D) across Caco-2 cells with (A, C) or without (B, D) change in ionic strength. The amounts of compounds permeated in the presence of 2.5 mM (○) or 125 mM (●) of potassium were measured. The concentrations of tryptamine and indoleacetic acid were 500 μM, and the pH of the apical buffer was 7.4. Osmolality was adjusted with D-mannitol (A, C; with change in ionic strength) or choline chloride (B, D; without change in ionic strength). Each point represents the mean of three measurements. Standard errors are within the range of the symbols.

layer. When the concentration of potassium ions was increased from 2.5 to 125 mM and the osmolality was adjusted with D-mannitol, that is, when the ionic strength was increased by the addition of potassium ions, the permeation rate of tryptamine decreased (Fig. 2A), whereas that of indoleacetic acid increased (Fig. 2C). On the other hand, when the osmolality was adjusted with choline chloride (i.e., when ionic strength did not change), there was no change in the permeation rate of either compound. These results suggest that a change in the transmembrane electric potential difference caused by potassium ions does not affect the permeation of the tested compounds, whereas a change in the ionic strength has significant effects.

3.4. Correlation between changes in ionic strength and permeation of tryptamine and indoleacetic acid through a Caco-2 cell monolayer

Fig. 3A and B shows the effects of ionic strength of the apical buffer on the permeation of tryptamine and indole-
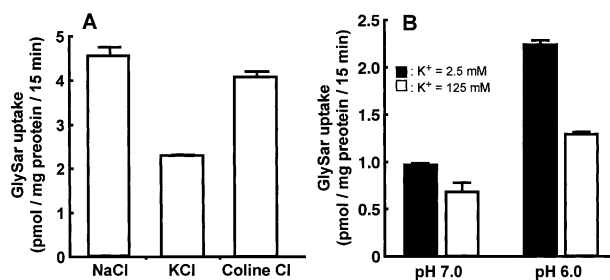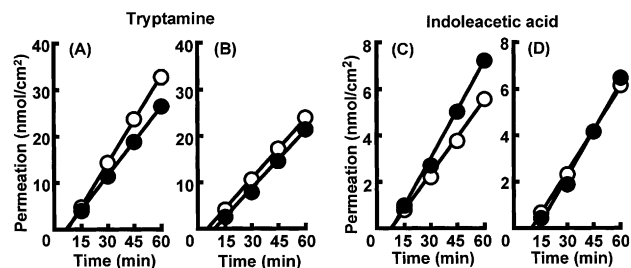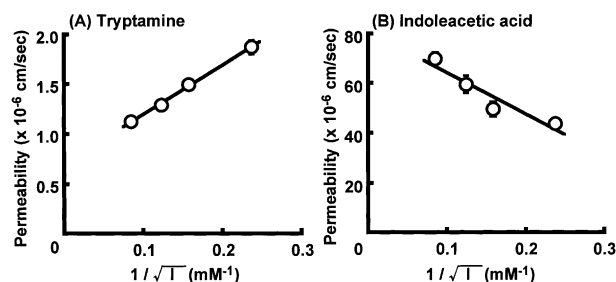

Fig. 1. Effect of potassium ion concentration on the uptake of glycylsarcosine by Caco-2 cells. (A) The composition of the uptake buffer was 25 mM Mes/Tris (pH 6.0) containing 140 mM NaCl (or KCl, choline Cl), 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, and 0.5 μM [^{3}H]glycylsarcosine. (B) The composition of the uptake buffer is shown in Table 1. The concentration of potassium was changed with potassium gluconate, and ionic strength was kept constant.

Fig. 3. Relationships between ionic strength (*I*) and permeability of tryptamine (A) and indoleacetic acid (B) across Caco-2 cells. The concentrations of tryptamine and indoleacetic acid were 500 μM. Ionic strength of the apical buffer varied with changes in the concentration of potassium gluconate (2.5, 25, 50, and 125 mM), and osmolality was adjusted by adding D-mannitol. Each point represents the mean ± S.E. of three measurements. Some of the standard errors are within the range of the symbols.
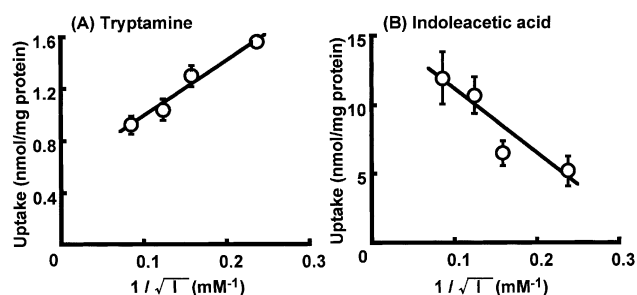
Fig. 4. Relationships between the uptake of tryptamine (A) and indoleacetic acid (B) and ionic strength of the apical buffer. The concentrations of tryptamine and indoleacetic acid were 500 μM. Ionic strength varied with changes in the concentration of potassium gluconate (2.5, 25, 50, and 125 mM), and osmolality was adjusted by adding D-mannitol. Each point represents the mean ± S.E. of three measurements.

acetic acid through a monolayer of Caco-2 cells. The ionic strength was varied with potassium gluconate, and the osmolality was compensated with D-mannitol. The permeation rate of tryptamine decreased with an increase in ionic strength, whereas that of indoleacetic acid increased. Fig. 4 shows the effects of ionic strength of the apical buffer on the uptake of tryptamine and indoleacetic acid by Caco-2 cells. The uptake of tryptamine decreased with an increase in the ionic strength (Fig. 4A). This result is consistent with our previous result showing a correlation between the uptake of tryptamine by rat intestinal BBM vesicles and changes in the ionic strength that can be explained by the Gouy–Chapman model. In this model, surface potential is inversely proportional to the square root of the monovalent salt concentration [2]. In contrast to that of tryptamine, the uptake of indoleacetic acid increased with an increase in the ionic strength (Fig. 4B). Since the surface potential of the intestinal BBM originates in the surface negative charge of the membrane, the increase in the uptake of indoleacetic acid may be due to a decrease in the electrostatic repulsion force between the membrane and indoleacetic acid. The surface negative charge also causes a decrease in local
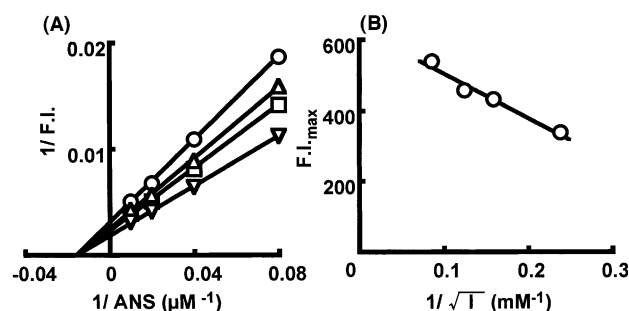

Fig. 5. (A) Double reciprocal plots of ANS concentration versus fluorescence intensity (F.I.) of ANS. Effect of ionic strength on the surface potential of Caco-2 BBM vesicles. Fluorescence intensities in the presence of 2.5 mM (○), 25 mM (△), 50 mM (□), and 125 mM (▽) of potassium gluconate. Osmolality was adjusted by adding D-mannitol. Each point represents the mean of three measurements. (B) Relationship between the ionic strength (I) of apical buffer and the maximum fluorescence intensity (F.I.$_{max}$) of Caco-2 BBM vesicles.

Table 2
n-Octanol-buffer distribution coefficient and permeability of cationic and anionic drugs

Cationic drug	log D_{oct}	Permeability		Decrease (%)
		2.5 mM K$^+$	125 mM K$^+$	
Atenolol	− 2.260	0.293 ± 0.008	0.093 ± 0.001	68.4
Chlorpromazine	2.212	6.771 ± 0.247	5.003 ± 0.243	26.1
Gramine	− 0.826	6.263 ± 0.123	4.599 ± 0.153	26.6
Imipramine	1.325	8.223 ± 0.226	6.052 ± 0.149	26.4
Metoprolol	− 0.800	4.472 ± 0.059	3.668 ± 0.018	18.0
Pheniramine	0.095	4.474 ± 0.066	3.573 ± 0.103	20.1
Phenylbutylamine	− 0.993	7.749 ± 0.224	5.542 ± 0.380	28.5
Pindolol	− 0.756	2.890 ± 0.039	2.018 ± 0.056	30.2
Ritodrin	− 0.407	0.332 ± 0.008	0.159 ± 0.003	52.2
Tryptamine	− 1.445	1.872 ± 0.067	1.124 ± 0.014	40.0

Anionic drug	log D_{oct}	Permeability		Increase (%)
		2.5 mM K$^+$	125 mM K$^+$	
Indoleacetic acid	0.000	43.5 ± 1.3	69.9 ± 1.5	60.7
Metiazic acid	1.823	89.7 ± 5.0	110.5 ± 3.4	23.2
Naproxen	1.557	106.5 ± 3.0	145.8 ± 6.4	36.9
Phenylacetic acid	− 0.187	83.9 ± 4.3	116.6 ± 8.0	39.1
Phenylbutylic acid	1.069	185.2 ± 4.2	236.0 ± 4.9	27.4
Salicylic acid	0.198	41.7 ± 1.6	75.8 ± 2.1	81.8
Tolmetin	1.394	46.2 ± 0.8	66.3 ± 0.4	43.5

Distribution coefficient (D_{oct}) was measured at pH 6.0 in the presence of 2.5 mM K$^+$. The unit of permeability is × 10^{-6} cm/s. Each value represents the mean ± S.E. of three measurements.

concentration of indoleacetic acid, which leads through the dissociation equilibrium, to a decrease in the local concentration of the unionized form of the drug which is likely to be the permeating species. The opposite effect will occur with cationic drugs. These results suggest that the permeation of tryptamine and indoleacetic acid through a monolayer of Caco-2 cells is also dependent on the membrane surface potential reflecting change in permeation through the BBM.

To confirm the changes in the membrane surface potential, we monitor the fluorescence intensity of ANS, a surface potential-sensitive probe [13–15] that binds to the BBM vesicles prepared from Caco-2 cells in the presence of various concentrations of potassium gluconate. The results are shown in Fig. 5. From the ordinate intercept of double reciprocal
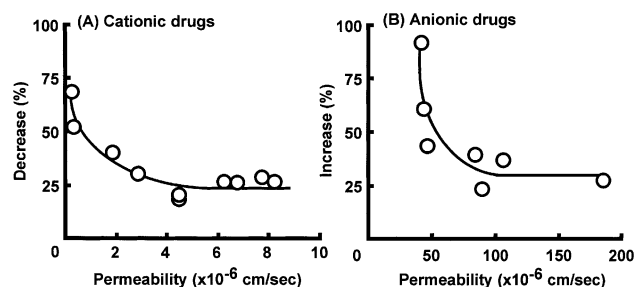

Fig. 6. Relationships between permeability across Caco-2 cells and effects of membrane surface potential on the transport of (A) cationic and (B) anionic drugs. Permeability in the presence of 2.5 mM potassium in Table 2 was used.

plots of fluorescence intensity against ANS concentration, maximum fluorescence intensity, $F.I._{max}$, was calculated (Fig. 5A). As shown in Fig. 5B, $F.I._{max}$ increased with increase in ionic strength. These results suggest that the change in the concentration of potassium gluconate in this study causes a significant change in the membrane surface potential.

Davis et al. [16] reported that ion pair formation with cationic ions, such as Na^+ and K^+, is a major mechanism of the good intestinal absorption of proxicromil, a strongly acidic antiallergy agent. They showed that the *n*-octanol-buffer distribution coefficients increased with increase in the cation concentration (ionic strength). On the other hand, Austin et al. [17] reported that ion pair formation was insignificant in the binding of proxicromil to dioleoylphosphatidylcholine liposomes even though *n*-octanol-buffer distribution coefficient was increased with increase of ionic strength. Moreover, Alcorn et al. [18] reported the results that did not support the concept of ion-pairing enhancing absorption via increased extraction into membranes. Furthermore, ion-pair formation cannot explain the decrease of the uptake or the permeation of tryptamine by an increase in ionic strength. Therefore, ion-pair formation is not likely the primary mechanism underlying the changes in the uptake and the permeation of these compounds.

3.5. Generality of the effect of ionic strength on the permeation of drugs

In order to determine the generality of the effect of ionic strength on the permeation of cationic and anionic compounds across a monolayer of Caco-2 cells, we measured the permeation rates of ten cationic compounds and seven anionic compounds. This transport study was carried out in the presence of 2.5 or 125 mM of potassium ions (i.e., two different ionic strength conditions). As shown in Table 2, the permeation rates of all of the tested cationic compounds decreased, whereas those of the anionic compounds increased. Moreover, no differences were found in the permeation rates of neutral (acetanilide, antipyrine, and caffeine) and zwitterionic (enoxacin) drugs in the presence of 2.5 mM and in the presence of 125 mM of potassium (not shown). These results suggest that decreases and increases in the permeation rates of cationic and anionic compounds due to changes in the ionic strength are common in the respective groups of drugs. The percentages of decreases in the permeation rates of cationic compounds and increases in the permeation rates of anionic compounds with increase in the concentration of K^+ from 2.5 to 125 mM are shown in Table 2. The degrees of the effects of ionic strength on the permeation rates of the tested compounds varied.

3.6. Relationship between effect of ionic strength and permeation rates of drugs

Fig. 6 shows the relationship between effects of ionic strength on permeation through the monolayer of Caco-2
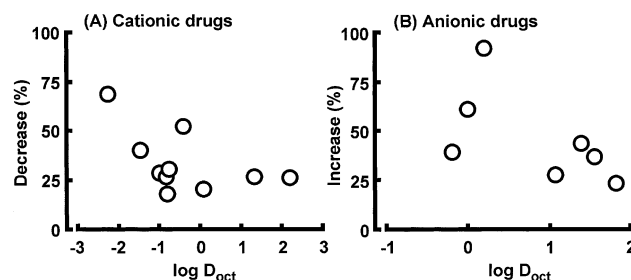

Fig. 7. Plots of *n*-octanol-buffer distribution coefficient versus the effect of membrane surface potential on the permeation of (A) cationic and (B) anionic drugs across Caco-2 cells.

cells (Table 2) and permeation rates of cationic (Fig. 6A) and anionic (Fig. 6B) compounds in the presence of 2.5 mM of potassium ions (Table 2). As shown in Fig. 6, the compound that showed a relatively lower permeation rate was given stronger effect. Fig. 7 shows plots of the decrease and increase in the permeation rate versus *n*-octanol-buffer distribution coefficient (D_{oct}) of the tested drugs (Table 2). There was no particular relationship except for a tendency for the effect on lopophilic drugs to be relatively small. In this study, we did not examine in further detail the relationships between physicochemical properties and permeation rates of the drugs.

In conclusion, the results in this study suggest that the electrostatic interaction caused by change in potassium ion concentration rather than by change in transmembrane electric potential difference is the dominant effect. The effects on the permeation rates of cationic and anionic drugs were opposite. We do not know the reason why the compound that showed a relatively lower permeation rate was given stronger effect. However, the possibility of overestimation or underestimation caused by these effects should be considered when the permeation of an ionic compound is evaluated using a cell monolayer system.

References

[1] M. Sugawara, M. Sasaki, K. Iseki, K. Miyazaki, Biochim. Biophys. Acta 1111 (1992) 145–150.
[2] M. Sugawara, H. Oikawa, M. Kobayashi, K. Iseki, K. Miyazaki, Biochim. Biophys. Acta 1234 (1995) 22–28.
[3] K. Iseki, M. Sugawara, N. Saitoh, K. Miyazaki, Biochim. Biophys. Acta 1146 (1993) 121–126.
[4] K. Iseki, M. Sugawara, N. Saitoh, K. Miyazaki, Biochim. Biophys. Acta 1152 (1993) 9–14.
[5] M. Sugawara, A. Hashimoto, T. Toda, M. Takahashi, M. Kobayashi, K. Iseki, K. Miyazaki, Biochim. Biophys. Acta 1190 (1994) 85–90.
[6] M. Sugawara, A. Hashimoto, M. Kobayashi, K. Iseki, K. Miyazaki, Biochim. Biophys. Acta 1192 (1994) 241–246.
[7] K. Iseki, K. Kaido, M. Kobayashi, M. Sugawara, K. Miyazaki, Biol. Pharm. Bull. 20 (1997) 794–799.
[8] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Gastroenterology 96 (1989) 736–749.
[9] P. Artursson, J. Karlsson, Biochem. Biophys. Res. Commun. 175 (1991) 880–885.

[10] K. Inui, H. Saito, M. Takano, T. Okano, S. Kitazawa, R. Hori, Biochim. Biophys. Acta 769 (1984) 514–518.

[11] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–275.

[12] Y.J. Fei, Y. Kanai, S. Nussberger, V. Ganapathy, F.H. Leibach, M.F. Romero, S.K. Singh, W.F. Boron, M.A. Hediger, Nature 368 (1994) 563–566.

[13] T. Aiuchi, N. Kamo, K. Kurihara, Y. Kobatake, Biochemistry 16 (1977) 1626–1630.

[14] J. Slavik, Biochim. Biophys. Acta 694 (1982) 1–25.

[15] T. Ohyashiki, M. Taka, T. Mohri, J. Biochem. 106 (1989) 584–588.

[16] M.G. Davis, C.N. Manners, D.W. Payling, D.A. Smith, C.A. Wilson, J. Pharm. Sci. 73 (1984) 949–953.

[17] R.P. Austin, P. Barton, A.M. Davis, C.N. Manners, M.C. Stansfield, J. Pharm. Sci. 87 (1998) 599–607.

[18] C.J. Alcorn, R.J. Simpson, D.E. Leahy, T.J. Peters, Biochem. Pharmacol. 45 (1993) 1775–1782.