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In vitro permeation of several model drugs across rabbit nasal mucosa

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Summary

The objectives in the present study were to establish a useful in vitro experimental method for detailed understanding of the nasal absorption of several drugs and its enhancement. Nasal mucosa was excised from the nasal septum in rabbits and mounted in a Ussing-type diffusion chamber. Firstly, the electrophysiological parameters, transmucosal potential difference, short-circuit current, and transmucosal electrical resistance (R_m) were measured to determine the mucosal viability. Viability was maintained over at least 6 h after mounting the mucosa in the chamber. Secondly, the in vitro membrane permeability of several model drugs was measured using the diffusion chamber. Disodium cromoglycate, fluorescein isothiocyanate-dextran (FD) of different molecular weights (4400, 9400, 35 600 and 71 200), and propranolol hydrochloride were chosen as model drugs. The membrane permeation profile showed a typical pseudo steady-state curve with a short lag time, and the permeability coefficient (cm/s) of these model drugs was calculated to be 3.428×10^{-6}, 1.275×10^{-6}, 0.677×10^{-6}, 0.181×10^{-6}, 0.126×10^{-6} and 2.554×10^{-5}, respectively. Thirdly, the effects of several permeation enhancers on the membrane permeation of FD (Mol. Wt 9400) as well as the electrophysiological parameters were evaluated. Four bile salts, namely, sodium glycocholate, sodium taurocholate, sodium deoxycholate (DC) and sodium taurodihydrofusidate (STDHF), were selected as permeation enhancers. Each enhancer rapidly reduced the R_m value, and hence increased the mucosal permeability of FD. The enhancing effect of DC and STDHF on FD permeation was greater than that of other two. This experimental method using the Ussing type diffusion chamber appears to be a useful tool for understanding the nasal absorption of drugs and the mechanism of absorption enhancement.

Introduction

Many investigators have focused on the nose as a desirable absorption site for peptide and protein drugs to avoid their first-pass effect or gastrointestinal degradation. Studies on the nasal absorption of drugs and the greater enhancement of their absorption have been reported using in vivo (Hussain et al., 1984; Hirai et al., 1981a; Shipper et al., 1990) and in situ (Huang et al., 1985; Gibson and Olanoff, 1987; Tengamnuay and Mitra, 1990) experimental techniques. A good in vitro system has been sought, however, in

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order to gain a detailed understanding of the nasal absorption mechanism and its enhancement, since they are difficult to determine by in vivo and in situ techniques. There have been recent reports on in vitro experiments using excised nasal mucosa (Hersey and Jackson, 1987; Wheatley et al., 1988; Uchida et al., 1991). The vital epithelial cell layer in this mucosa appears to play an important role as a barrier to drug permeation, thus making maintenance of the viability of excised mucosa throughout an in vitro experiment essential. Some of the earlier in vitro studies, however, did not take heed of this. In this study, we chose a Ussing type diffusion chamber (Kimura et al., 1982) to establish a useful in vitro experimental method to aid in our understanding of the nasal absorption of several drugs and the factors responsible for their enhancement. The viability and barrier function of the nasal mucosa excised from rabbit were checked by monitoring the electrophysiological parameters (Moore et al., 1989) of transmucosal potential difference (PD), short-circuit current (I_{sc}) and transmucosal electrical resistance (R_m). Disodium cromoglycate (DSCG), fluorescein isothiocyanate-dextran (FD) of different molecular weights (4400, 9400, 35 600, and 71 200) and propranolol hydrochloride (PROP · HCl) were chosen as model drugs and their permeabilities were followed using the diffusion technique. The enhancing effect of four bile salts, namely, sodium glycocholate (GC), sodium taurocholate (TC), sodium deoxycholate (DC) and sodium taurodihydrofusidate (STDHF), on the membrane permeation of FD (Mol. Wt 9400) was also evaluated, and the utility of the Ussing chamber system was discussed from the point of view of both permeation profiles of the drugs and the electrophysiological parameters of the nasal mucosa.

Materials and Methods

Materials

Model drugs DSCG and FD of different molecular weights (4400, FD4; 9400, FD10; 35 600, FD40; 71 200, FD70) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). PROP · HCl was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

Permeation enhancers GC and TC were obtained from Sigma Chemical Co.. DC was purchased from Tokyo Kasei Industries (Tokyo, Japan). STDHF was generously supplied by Leo Pharmaceuticals (Bullerup, Denmark). Other chemicals were of reagent grade. All reagents were used without further purifications.

Tissue preparations

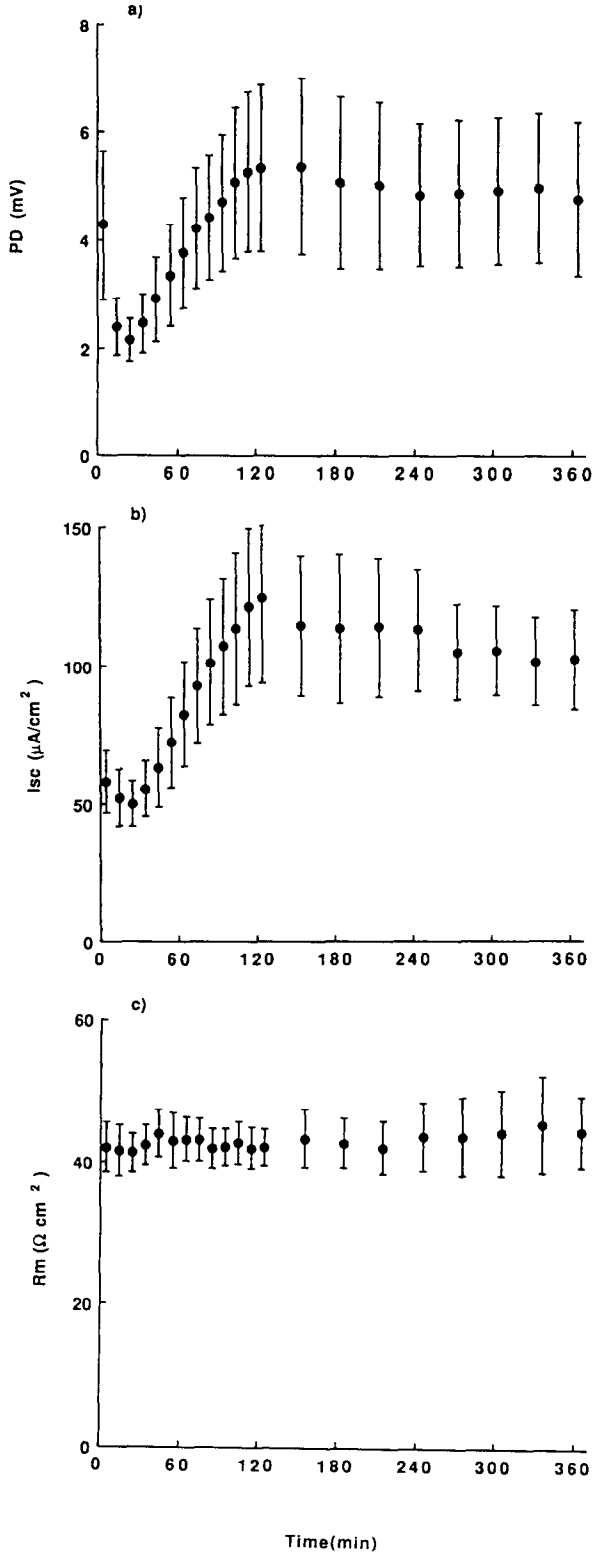
The nasal mucosa used in these experiments was obtained from a male Japanese white rabbit weighing between 2.0 and 3.0 kg (Tokyo Laboratory Animals Co., Tokyo, Japan). The rabbit was killed by rapid air embolism. The nasal septum was carefully removed from a bone block and placed in ice-cold Ringer solution (NaCl, 125 mM; KCl, 4.9 mM; $CaCl_2$, 1.4 mM; $NaHCO_3$, 10 mM; NaH_2PO_4, 1.2 mM; D-glucose, 11.1 mM) after adequate rinsing with Ringer solution. Two pieces of nasal mucosa were then carefully stripped from the nasal septum without touching the center of surface. The tissue was mounted in a 0.5 cm^2 oval window with a rubber O-ring between two Lucite disks set in a Ussing type diffusion chamber. Ringer solution (11 ml) was added to both chambers (mucosal side and serosal side) and oxygenated (95% O_2/5% CO_2). The entire system was maintained at 37°C throughout the experiment.

Electrophysiological parameters

PD and I_{sc} were measured between two salt bridges (3% agar in 150 mM NaCl solution) connected via each electrode to a short circuit amplifier (model CEZ-9100, Nihon Kohden Industries, Tokyo, Japan). R_m was calculated from the PD and I_{sc} values according to Ohm's law.

Permeation studies

Before permeation experiments, the nasal mucosa prepared in the above manner was equilibrated in Ringer solution for 120 min to achieve an electrophysiological steady state. A test solution containing a model drug (DSCG 5.1 mg/ml, FD 2.5 mg/ml or 5.0 mg/ml, PROP · HCl 1.0 mg/ml) was then introduced into the mucosal



side with or without permeation enhancer. 1 ml samples were taken from the serosal side intermittently, and the same volume of fresh Ringer solution was added to keep the volume constant. Flux of the drug was calculated from the increase in concentration on the serosal side over time.

Analysis

A high-performance liquid chromatography system consisting of a pump (LC-6A, Shimadzu, Kyoto, Japan), an ultraviolet detector (SPD-6A, Shimadzu), an integrator (CR-6A, Shimadzu) and a column (Capcellpack C18, Shiseido, Tokyo, Japan) was used for analyzing the DSCG concentration at a wavelength of 329 nm. The fluorescence intensity of FD and PROP was measured with a fluorescence spectrophotometer (RF-5000, Shimadzu) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm, and at 295 and 360 nm, respectively.

Morphological assessment

After the permeation study, the nasal mucosa was fixed in isotonic formalin solution (10% formalin in physiological saline) at 5°C for 12 h. The specimen obtained was dried by gradually immersion in ethanol/water (from 50 to 100%) and a critical-point drier (HCP-2, Hitachi, Tokyo, Japan). The specimen was finally coated with gold for examination with a scanning electron microscope (X-650, Hitachi).

Results

Basic electrophysiological parameters

Fig. 1 shows the basic electrophysiological parameters of rabbit nasal mucosa. These were equilibrated within 90–120 min after setting up the membrane in the chambers, and then pseudo steady-state values were stably maintained for at least 4 h. Although the electrophysiological properties observed in the rabbit nasal mucosa were

Fig. 1. Basic electrophysiological parameters of the rabbit nasal mucosa: (a) PD, (b) I_{sc}, (c) R_m. Each point represents the mean ± S.E. ($n = 5$).

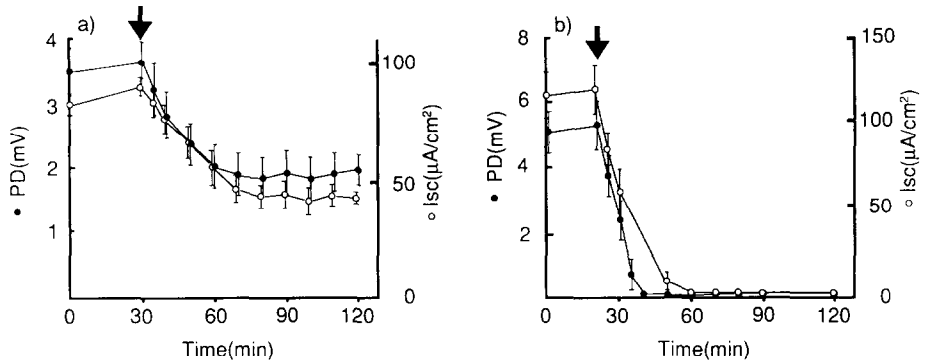


Fig. 2. Effect of glucose-free and oxygen-free conditions on PD and I_{sc}: (a) glucose-free, (b) oxygen-free. Each point represents the mean ± S.E. ($n = 3$). Arrow shows change to glucose-free or oxygen-free condition.

inconsistent with those in sheep, the period to equilibrium was almost the same (Wheatley et al., 1988). Therefore, the equilibrium time was set at 120 min.

Effect of glucose-free and oxygen-free conditions

Fig. 2 shows the effect of glucose-free Ringer solution (11.1 mM D-mannitol instead of D-glucose) and nitrogen (instead of oxygen) on the electrophysiological parameters of the nasal mucosa. Glucose-free and oxygen-free conditions reduced both PD and I_{sc} values, which indicated destruction of the viability of the nasal mucosa under these conditions. Therefore, a combination of Ringer solution containing D-glucose and oxygen bubbling is essential to retain the membrane viability.

Effect of 2,4-dinitrophenol and ouabain

Fig. 3 shows the effect of 2,4-dinitrophenol (DNP) and ouabain, which have been used as an uncoupler (Madara et al., 1987) and a Na^+/K^+-ATPase inhibitor (Smith et al., 1988), respectively, on the electrophysiological parameters of the nasal mucosa. Addition of DNP (1 mM) to both sides (mucosal and serosal sides) of the chamber rapidly reduced the PD and I_{sc} value (Fig. 3a). The presence of ouabain (1 mM in the serosal side) to block Na^+/K^+-ATPase localized in the basolateral membrane (Ducroc et al., 1983) also lowered the PD and I_{sc} value to almost 0 mV and 0 $\mu A/cm^2$ within 10 min (Fig. 3b). These results indicated that PD and I_{sc} were produced by Na^+/K^+-ATPase and that the values were dependent on metabolic energy. It is

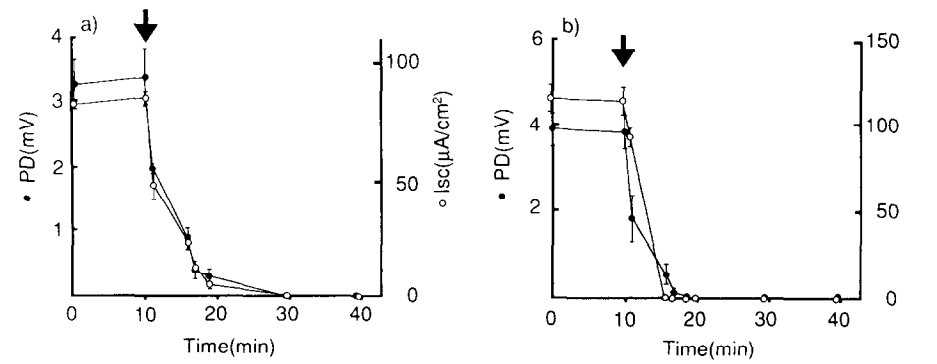


Fig. 3. Effect of DNP (1 mM) and ouabain (1 mM) on PD and I_{sc}: (a) DNP, (b) ouabain. Each point represents the mean ± S.E. ($n = 3$). Arrow shows addition of DNP (to mucosal and serosal sides) or ouabain (to serosal side).

clear from an electrophysiological point of view that tissue viability can be retained in our experimental system.

Permeation profile of model drugs through the nasal mucosa

Fig. 4 shows the permeation profile of model drugs through the nasal mucosa. The flux of all drugs showed a typical pseudo steady-state curve with a short lag time and was almost constant over 4 h. The permeability coefficients (cm/s) of DSCG, FD10 and PROP · HCl were calculated to be 3.428×10^{-6}, 0.677×10^{-6} and 2.554×10^{-5}, respectively. With DSCG and FD10, little morphological alteration in the cilia was observed compared to control (Fig. 5b and c) and no changes were observed in the electrophysiological parameters (data not shown). On the other hand,

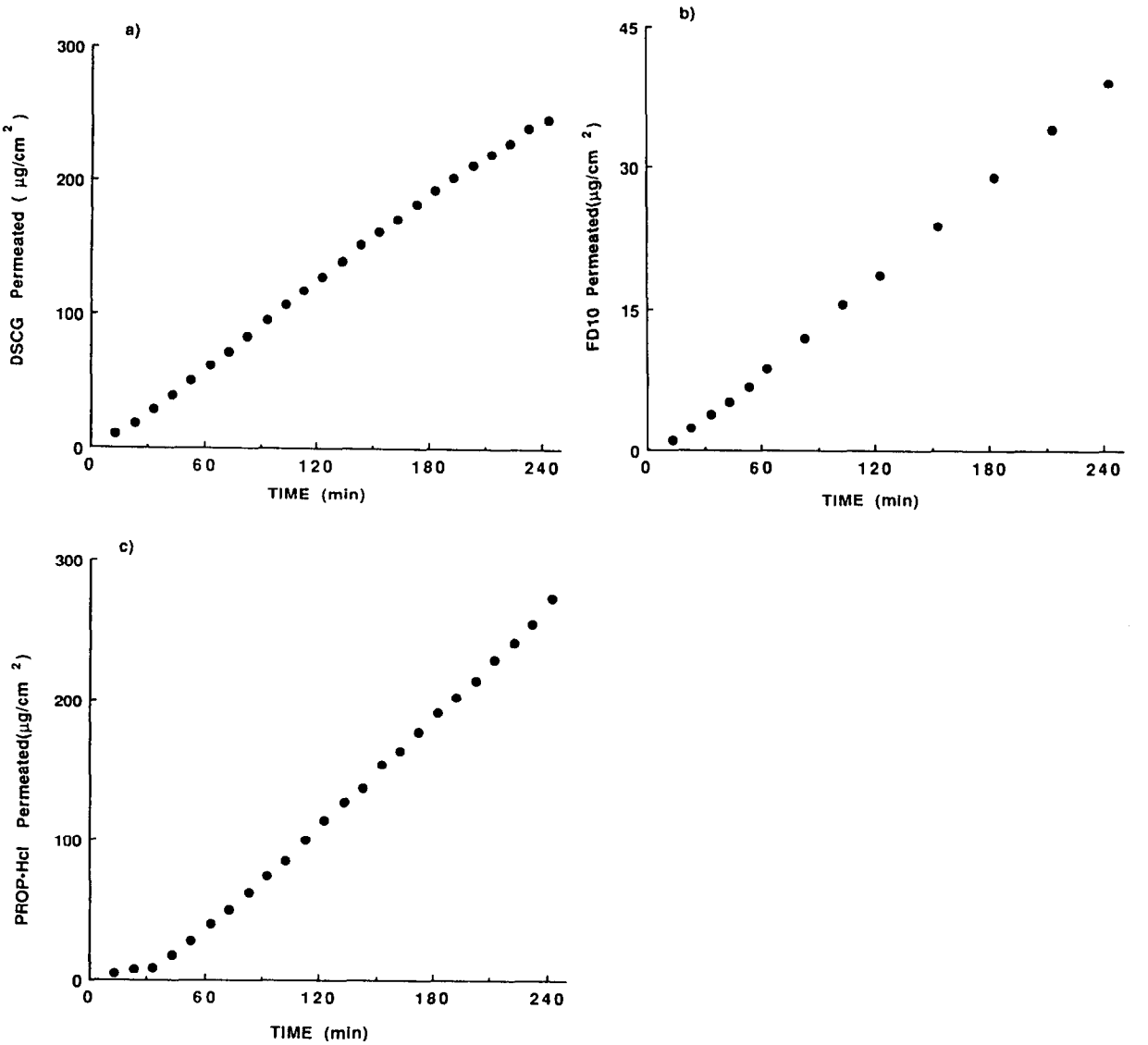


Fig. 4. Permeation profile of model drugs across the rabbit nasal mucosa: (a) DSCG (5.1 mg/ml), (b) FD10 (5.0 mg/ml), (c) PROP · HCl (1.0 mg/ml).

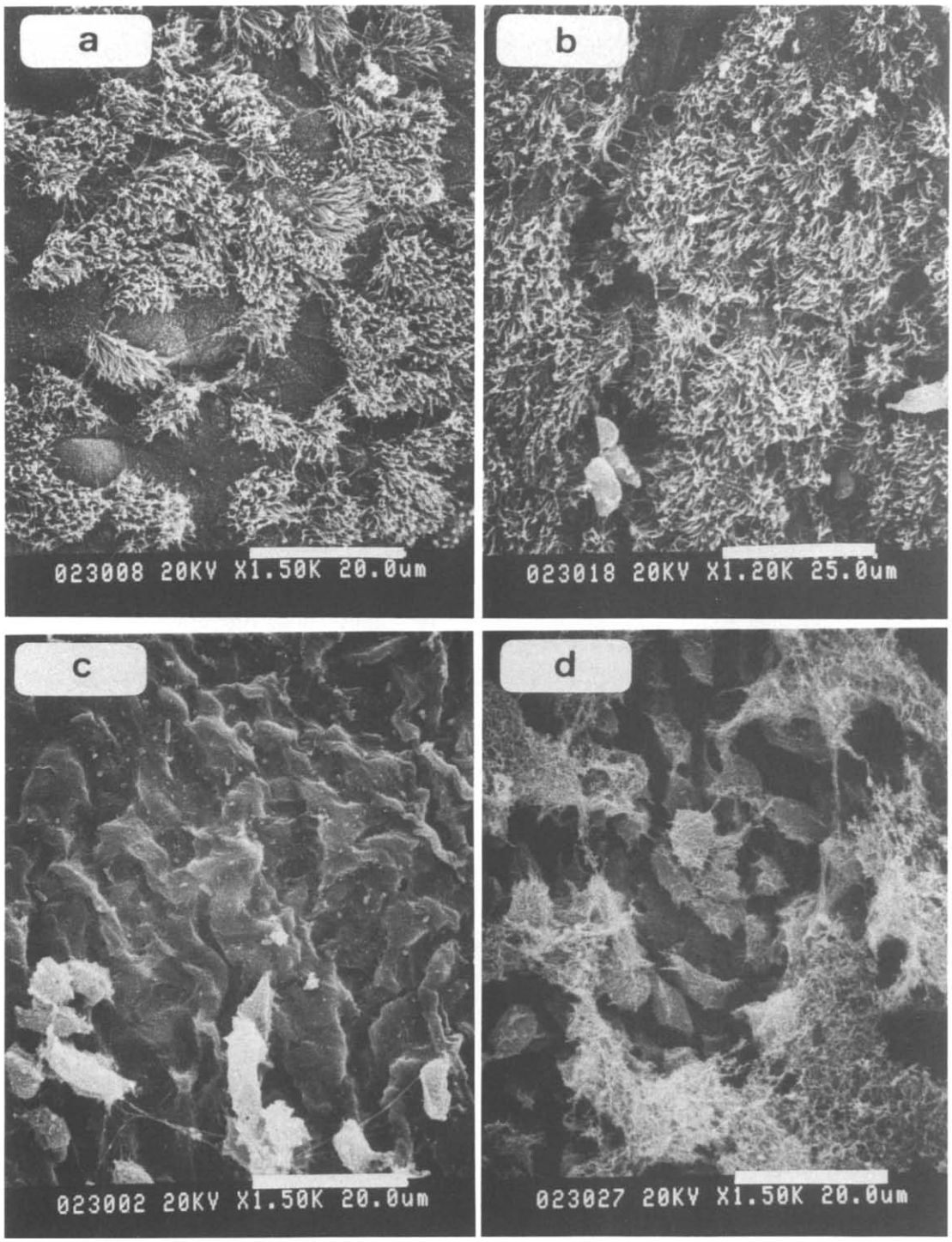


Fig. 5. Scanning electron micrographs of mucosal surface after permeation studies of model drugs. (a) Control (after incubation in Ringer solution for 6 h), (b) DSCG (5.1 mg/ml), (c) FD10 (5.0 mg/ml), (d) PROP · HCl (1.0 mg/ml).

1 mg/ml of PROP · HCl rapidly reduced PD and I_{sc} (data not shown) and changed the morphology of the mucosal surface (Fig. 5d). These results appear to indicate that DSCG and FD lack a significant effect on the morphology or integrity of nasal mucosa, whereas PROP · HCl does exert an appreciable influence.

A good linear correlation was found between the log permeability coefficient and the log molecular weight, except for PROP · HCl (Fig. 6). The permeation of PROP · HCl clearly was not dependent on the physicochemical property of molecular weight but rather on morphological changes in the mucosal surface.

Effect of bile salts

The permeation of FD10 increased and the R_m value decreased after addition of 5 mg/ml of GC, TC, DC and STDHF (Fig. 7). The enhancing effects of DC and STDHF on FD10 permeation were greater than those of GC and TC, as was the degree of decrease in R_m. The increase in FD10 permeation thus seems to reflect the decrease in R_m. Fig. 8 shows the mucosal surface morphology after a permeation study to detect the effect of each bile salt (Fig. 8a–d correspond to Fig. 7a–d, respectively). With DC (Fig. 8c), there was a considerable change in mucosal surface morphology (the epithelial cells were completely denuded). Exposure to STDHF changed the cilia and caused partial denudation of epithelial cells, while mild morphological changes on mucosal surface were observed with GC and TC. In addition, the decrease in R_m and morphological change shown in Figs 7 and 8 were also observed after exposure to each bile salt solution without FD10 (data not shown).

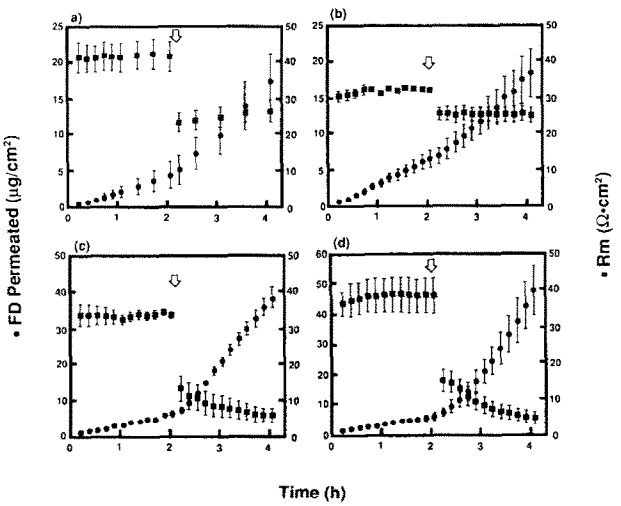


Fig. 7. Effect of bile salts on permeation of FD10 and R_m: (a) GC (5 mg/ml), (b) TC (5 mg/ml), (c) DC (5 mg/ml), (d) STDHF (5 mg/ml). Arrow shows replacement of FD10 solution to FD10 solution with bile salt. Each point represents the mean ± S.E. ($n = 3$). Concentration of FD10 was 2.5 mg/ml.

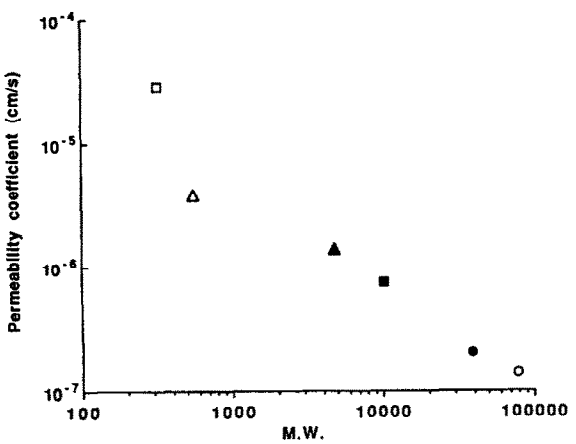


Fig. 6. Relationship between molecular weight and permeability coefficient: (□) PROP·HCl (1 mg/ml), (△) DSCG (5.1 mg/ml), (▲) FD4 (5.0 mg/ml), (■) FD10 (5.0 mg/ml), (●) FD40 (5.0 mg/ml), (○) FD70 (5.0 mg/ml).

Discussion

Efforts to clarify the drug absorption and absorption enhancing mechanism of nasal mucosa have been made by many investigators. Some information was provided by in situ circulation experimental systems (Hirai et al., 1981b). Nevertheless, a well-designed in vitro experimental system may be more advantageous to identify the mechanism. Ussing type diffusion chambers have been used to investigate the drug absorption mechanism across gastrointestinal mucosae (Munck and Rasmussen, 1979). Recently, Hersey and Jackson (1987) and Wheatley et al. (1988) reported that this technique could also be used

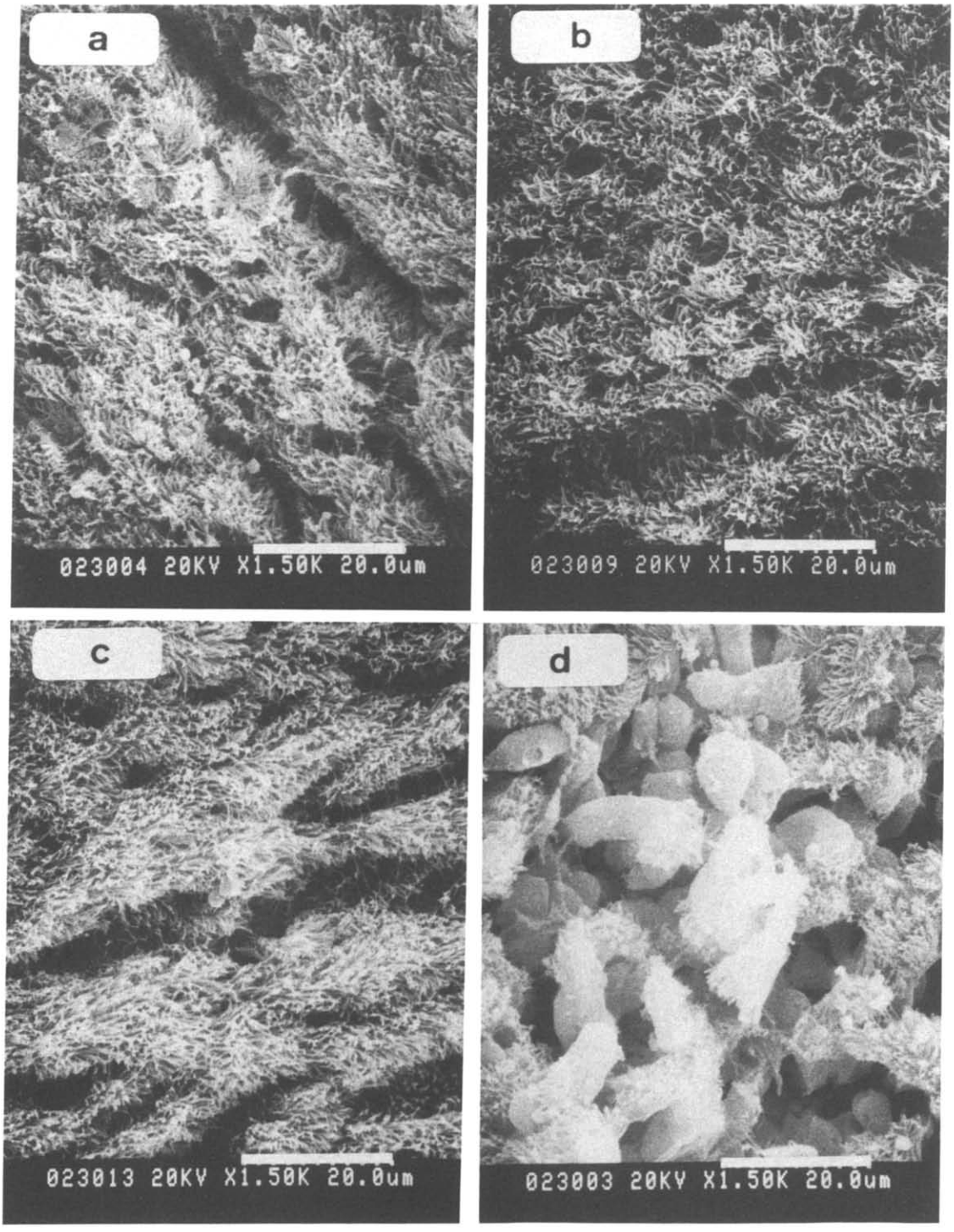


Fig. 8. Effect of bile salts on mucosal surface morphology: (a) GC (5 mg/ml), (b) TC (5 mg/ml), (c) DC (5 mg/ml), (d) STDHF (5 mg/ml). After permeation study of FD10 for 2 h, each sample was exposed to bile salt solution containing FD10 (2.5 mg/ml) for 2 h.

for in vitro nasal absorption of drugs, however, there little information is available about the relationship between the change in drug permeation profile and that of the electrophysiological parameters. In vitro experiments require the viability of the nasal mucosa to remain stable over the period of the experiment because epithelial cell layers act as a barrier to drug permeation. The Ussing chamber system makes it possible to perform detailed in vitro nasal absorption experiments. Ringer solution and oxygen bubbling were employed to keep the membrane viable over 6 h (Fig. 2). DNP and ouabain, viability inhibitors, markedly reduced PD and I_{sc}, suggesting that electrophysiological parameters had a major impact on the tissue viability. Monitoring of these parameters can be carried out by the Ussing chamber system as well as by determination of the transmucosal flux of a drug.

The PD and I_{sc} values at steady state obtained in rabbit were somewhat lower than those in sheep (12–15 mV, 100–200 $\mu A/cm^2$, respectively), as reported by Wheatley et al. (1988), perhaps due to inter-animal variation. Changes in R_m can be used as a parameter corresponding to drug permeation based on a structural change of the tight junction in rat jejunal mucosa (Yamashita et al., 1990). The R_m of the rabbit nasal mucosa was calculated to be approx. 40 Ω cm^2 under the control condition (Fig. 1). PROP · HCl and bile salts reduced R_m and caused morphological changes, and the latter enhanced the permeability of FD10. These results suggested that R_m can be used to evaluate the permeability of drugs.

In this study, DSCG, FD and PROP · HCl were chosen as hydrophilic, hydrophilic-macromolecular and lipophilic drugs, respectively. DSCG and FD had no effect on the electrophysiological parameters (data not shown) or on the mucosal surface morphology (Fig. 5). Together with steady-state flux (Fig. 4), these model drugs appeared not to influence the mucosal integrity. PROP · HCl (1 mg/ml), on the other hand, rapidly reduced the PD and I_{sc} (data not shown), and caused partial denudation of epithelial cells (Fig. 5d). Donk and Merkus (1982) pointed out that PROP · HCl indicated ciliotoxicity to the tracheal epithelium at a concentration of 0.1% from the point of view of ciliary beat frequency. Our in vitro data are similar to their results. R_m could therefore be used as an indication of mucosal damage after nasal drug administration, since functional and morphological damage of the nasal mucosa altered the electrophysiological parameters, especially R_m. This experimental system can also be useful to estimate irritation of a drug and adjuvant on the mucosa.

The molecular weight of a drug absorbed effectively from the nasal cavity without any adjuvant was reported to be lower than 1000 (McMartin et al., 1987). Fisher et al. (1987) also reported the effect of molecular size on the nasal absorption of water-soluble compounds (Mol. Wt 190–70 000) in rats. They indicated a good linear correlation between the log molecular weight and log intranasal absorption ratio. This chamber system could also be used to create a criterion for intranasally absorptive drugs, since our in vitro data (Fig. 6) are in good agreement with their results.

Ennis et al. (1990) reported that dihydroxy bile salts caused more severe morphological damage than trihydroxy bile salts; moreover, they enhanced intranasal absorption of insulin more than trihydroxy bile salts (Gordon et al., 1985); our in vitro data were consistent with their results. Coleman et al. (1976) reported that the dihydroxy bile salt, DC, (0.5%) completely dissolved erythrocyte ghost membranes due to solubilization of phospholipid and protein, while trihydroxy bile salts, GC and TC (0.5%) produced less effect. Hydrophobicity of bile salt was suggested by Gordon et al. (1985) to be an important factor in acting as an adjuvant. They showed that the adjuvant potency for nasal absorption of insulin positively correlated with the hydrophobicity of bile salts. The permeation enhancement of FD10 by bile salts shown in Fig. 7 might depend on the degree of morphological damage based on their hydrophobicity.

The Ussing chamber system is thus a very promising tool for use in studying the permeation of drugs across the nasal mucosa and for estimating the effect of permeation enhancers. The chamber can also check physiological and mor-

phological changes of the nasal mucosa by monitoring electrophysiological parameters.

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