International Journal of Pharmaceutics, 74 (1991) 95–103
Elsevier Science Publishers B.V.
ADONIS 037851739100279D

IJP 02472

Influence of bile salts on the permeability of insulin through the nasal mucosa of rabbits in comparison with dextran derivatives

Naoki Uchida, Yoshie Maitani, Yoshiharu Machida, Masayuki Nakagaki and Tsuneji Nagai

Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142 (Japan)

(Received 17 October 1990)
(Modified version received 20 February 1991)
(Accepted 2 March 1991)

Key words: Bile salt; Nasal absorption; Nasal mucosa; Dextran derivative; Insulin; Permeability coefficient

Summary

Nasal drug absorption and the effect of absorption promoters have been studied in rabbits. Nasal mucosa excised from rabbits was mounted as a flat sheet in an in vitro chamber. The increase in the membrane permeability coefficient for sodium chloride indicates the change in the porosity by pretreatment with bile salts. The permeabilities for dextran derivatives were enhanced by pretreatment with sodium glycocholate (GC). The permeability coefficient (P) for fluorescein isothiocyanate diethylaminoethyl dextran (FITC DEAE-dextran, DE) was higher than that for FITC-dextran (DT), and P for FITC dextran sulfate (DS) was lower than that for DT with the same molecular weight. Comparing insulin with dextran of the same molecular weight as insulin, the value of P for insulin induced by pretreatment with GC was higher than that for hydrophilic dextran. The partition coefficient seemed to have much more effect on the nasal membrane transport than the molecular weight of the penetrant.

Introduction

Peptides are usually inactivated when administered orally, due to their instability against various peptidases in the gastrointestinal tract. Therefore, intranasal administration has been investigated as a new route for systemic peptide delivery (Chien, 1985). However, there have been few reports on the physicochemical properties of nasal mucosa in experimental animals (Wheatley et al., 1988). Understanding of membrane permeability will lead to a more effective way for delivery of drugs. Previously, we had measured the membrane potential of the nasal mucosa that is an important factor in drug permeation of the nasal mucosa in rabbits (Maitani et al., 1991). The nasal mucosa is negatively charged and the membrane charge densities are not changed by pretreatment with 1% bile salt solution on the mucosal side. In nasal absorption of peptide, bile salts are used as absorption promoters (Hirai et al., 1981; Maitani et al., 1988). However, the mechanism by which bile salts enhance membrane permeability is not known clearly. The bile salts might affect the nasal membrane and create

Correspondence: Y. Maitani, Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan.

temporal pores (Gordon, 1985). Therefore, the effect of bile salt on the nasal membrane was evaluated by enhancement of the membrane permeability coefficient of sodium chloride caused by pretreatment with bile salts. Also, the permeabilities for dextran derivatives with molecular weights ranging from 3860 to 40500 were measured to investigate the action of bile salt on the pore size of the membrane examined.

In addition, the bile salt might transiently modify membrane charges and increase transport of the charged compound. Therefore, the permeabilities of three dextran derivatives which represent neutral, positive and negative charges and of insulin were also investigated.

Materials and Methods

Materials

The chemicals used were as follows: fluorescein isothiocyanate (isomer-1, FITC) was obtained from Wako Pure Chem. Ind. Ltd; sodium cholate (C) from Tokyo Kasei Ind. Ltd; ethylenediamine tetraacetic acid, and disodium salt (EDTA) from Iwai Chem. Co.; sodium deoxycholate (DC) and sodium glycocholate (GC) from Nakarai Chemical Co.; dextran (mw. 6000, 9000), FITC-dextran (DT, mw. 3860, 9000, 17500, 40500), dextran sulfate (mw. 5000, 8000), crystalline bovine pancreas insulin (24.5 international units (IU) per mg; zinc content, approx. 0.5%), sodium glycodeoxycholate (GDC), sodium taurocholate (TC) and sodium taurodeoxycholate (TDC) from Sigma Chemical Co. (U.S.A.). Sodium chloride was purchased from Matsunaga Chem. Ind. Ltd and used after drying under vacuum at 120 °C for 6 h. All other chemicals used were analytical grade. All solutions were made using doubly distilled water in Pyrex glass.

Preparation of DEAE-dextran, FITC-labeled DEAE-dextran (DE) and FITC-labeled dextran sulfate (DS)

Diethylaminoethyl dextran (DEAE-dextran) was synthesized according to the method reported by McKernan and Ricketts (1960). DEAE-dextran (mw. 6000) and DEAE-dextran (mw. 9000) were obtained, the N content being determined by elemental analysis as 0.55% and 0.50%, respectively. The average number of basic groups per glucose unit of DEAE-dextrans (mw. 6000, 9000) was calculated from the N content as 0.075 and 0.068 basic group per glucose unit, respectively.

Fluorescein isothiocyanate-labeled DEAE-dextran (DE, mw. 6000, 9000) and fluorescein isothiocyanate-labeled dextran sulfate (DS, mw. 5000, 8000) were synthesized from DEAE-dextran and dextran sulfate, respectively, according to the method reported by Belder and Granath (1973). The method for synthesis and molecular weight determination was described precisely in a previous paper (Maitani et al., 1989).

Setting of nasal mucosa

The nasal mucosa used in these experiments was obtained from male New Zealand white rabbits (Saitama Experimental Animal Supply Co.; 2.5–3.0 kg). Rabbits were killed by rapid i.v. injection of a saturated solution of potassium chloride. The nasal mucosa was obtained by cutting a bone block, with operating scissors, from orbits just anterior to the junction of the nasal bone with the dorsal parietal cartilage. After dissection, the tissue was rinsed in saline solution and any adhering cartilage and blood were removed. After rinsing in distilled water, a piece of tissue was mounted as a flat sheet in a 15.2 mm^2 circular window between two glass disks that was set between two glass chambers with the aid of silicone O-rings to prevent the solution from leaking into the chambers. The thickness of the membrane was 0.07–0.40 mm, being measured with a dial thickness gauge having an accuracy of ± 0.001 mm.

Permeability measurement for sodium chloride

Fig. 1 shows the apparatus used for measurement of NaCl permeability. The donor cell, the membrane with glass disks and the receiver cell were assembled together with springs. 40 ml each of NaCl aqueous solution (2×10^{-1} M) and distilled water were put into the donor (mucosal) and the receiver (serosal) chambers, respectively. The mucosal surface (M) which is the ciliated

epithelial surface lining the nasal cavity and the serosal surface (S) which is the side attached to the nasal cartilage were exposed to the donor side and receiver side, respectively. The conductivity of the receiver solution was measured every 2 min for 30 min with platinum electrodes inserted in the receiver chamber to calculate the concentration of NaCl. After the measurement, the donor solution was changed to 40 ml of bile salt aqueous solution (10 mM) and kept for 5 min; then the bile salt solution was replaced again with NaCl aqueous solution after rinsing with distilled water. Also, the receiver solution was replaced with a fresh solution. The NaCl permeability after the pretreatment with bile salts was measured according to the method described above. In this study, all experiments were conducted at $36 \pm 0.2\,^{\circ}C$.

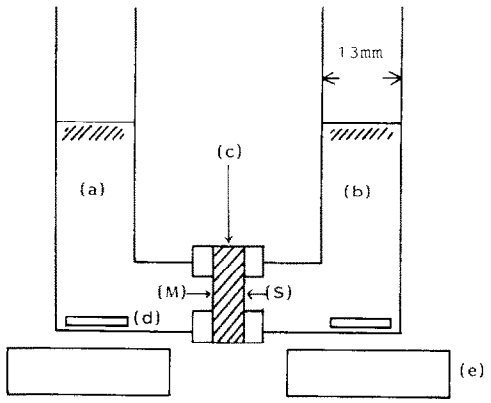

Fig. 2. Apparatus for measurement of permeability for dextran derivatives and insulin. (a), donor cell (mucosal chamber); (b), receiver cell (serosal chamber); (c), nasal mucosa (M, mucosal side; S, serosal side); (d), stirrer bar; (e), magnetic stirrer.

Permeability measurement for dextran derivatives

Fig. 2 shows the apparatus used for measurement of the permeability for dextran derivatives. The volume of both donor and receiver cells which were held together by springs was 10 ml. The apparatus was immersed in a water bath maintained at $36 \pm 0.2\,^{\circ}C$. In the permeability measurement for dextran derivatives, 6 ml each of the following solutions were used as the donor solutions; 0.01% DT in saline solution, 0.01% DE in saline solution, 0.1% DS (mw. 5000) in saline solution, 1% DS (mw. 8000) in saline solution. A 6 ml saline solution was used for the receiver solution. A 200 μl portion of receiver solution was withdrawn as the sample solution at 10 min intervals for 60 min and immediately replaced with an equal volume of saline solution. The donor solution was removed from the chamber, and then 6 ml of GC aqueous solution (10 mM) was added to the donor chamber. After 5 min, the GC solution was replaced with the initial donor solution after rinsing with distilled water. Additionally, the receiver solution was replaced with fresh solution. The permeability for dextran derivatives after pretreatment by GC was measured according to the method described above. The fluorescence intensity of the dextran derivative was measured with a fluorescence spectrophotometer (Shimadzu, model RF-530) at an emission wavelength of 516 nm and an excitation wavelength of 480 nm. The quantity of the dextran derivative in the sample solution was calculated from the fluorescence intensity relative to that of a standard solution of the dextran derivative.

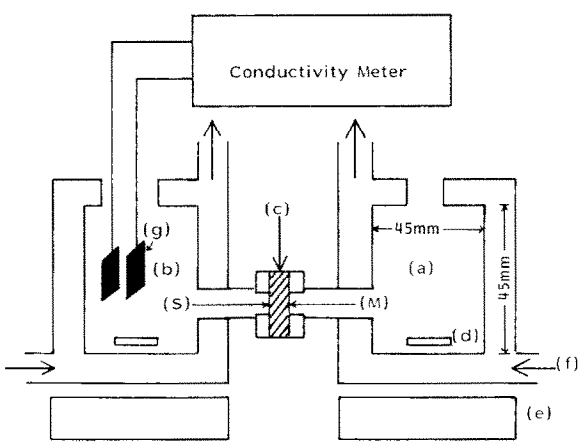

Fig. 1. Apparatus for measurement of membrane permeability for NaCl. (a), donor cell (mucosal chamber); (b), receiver cell (serosal chamber); (c), nasal mucosa (M, mucosal side; S, serosal side); (d), stirrer bar; (e) magnetic stirrer; (f), water bath; (g), platinum electrode.

Permeability measurement for insulin

In the measurement of permeability for insulin, 6 ml samples of 10 IU/ml insulin saline solutions (pH 2.0 and pH 5.4) were used as donor solution with the same apparatus as shown in Fig. 2. A 6 ml portion of the saline solution was used for the receiver solution. The insulin solution (pH 5.4) was prepared by dissolving insulin first in 0.2 ml of 0.1 N hydrochloric acid, and then by dilution with the saline solution. The pH of the insulin solution was adjusted to pH 2.0 using hydrochloric acid. A 300 μl portion of the receiver solution was withdrawn as the sample solution at 10 min intervals for 60 min. The donor solution was then removed from the chamber and 6 ml of GC aqueous solution (10 mM) was added in the donor chamber and kept for 5 min. The GC solution was then replaced with the insulin saline solution after rinsing with distilled water. Also, the receiver solution was replaced with fresh solution. The permeability for insulin after the pretreatment with GC was measured according to the method described above. The insulin concentration was measured by using enzyme immunoassay with a commercially available kit (Insulin EIA kit, Dainabot Co. Ltd).

Theory

The concentration (C) of NaCl in the receiver solution (serosal chamber) was plotted as a function of time (t). From the slope, the amount of NaCl permeated per unit time and area (A), i.e., flux (J) was calculated. The permeability coefficient (P) is the apparent permeability coefficient (P′) multiplied by the thickness of the membrane (ΔX). The experimental value of P′ was calculated according to Eqn 1:

$$J = (dC/dt) \cdot V/A = P' \cdot C_D \qquad (1)$$

where C_D is the concentration of NaCl in the donor solution (mucosal chamber), and V is the volume of the receiver solution. The permeability coefficient, P, can be represented as Eqn 2 (Nakagaki et al., 1962):

$$P = f \cdot \beta \cdot \phi \cdot D_0 \qquad (2)$$

where f is the membrane constant, β is the partition coefficient between the solution and the membrane layer, D_0 is the diffusion coefficient in water, and ϕ is (D^*/D_0) when the diffusion coefficient in the membrane is D^*. In this case, the partition coefficient β is affected Donnan equilibrium, and the value of β varies with the concentration of the electrolyte. The concentration dependence of P is therefore considered to be ascribed primarily to that of the partition coefficient β. The membrane permeability coefficient (P^*) is calculated according to Eqn 3:

$$P^* = (J \cdot \Delta X)/C^* = f \cdot \phi \cdot D_0 \qquad (3)$$

where C^* is the concentration of NaCl in the mucosal layer of the membrane (donor cell), and is given in Eqn 4 (Kobatake et al., 1965):

$$C^* = \left(-\theta^* + \sqrt{\theta^{*2} + 4C^2}\right)/2 \qquad (4)$$

where θ^* is the membrane charge density of the nasal mucosa in NaCl solution. θ^* was previously calculated by measurement of the membrane potentials of the nasal mucosa in rabbits ($\theta^* = -3.5$ mM/l) (Maitani et al., 1991). The value of C^* is obtained from Eqn 4 and then the value of P^* is also obtained. The membrane permeability coefficient after the pretreatment with bile salt aqueous solution (P_t^*) can be represented as Eqn 5:

$$P_t^* = f_t \cdot \phi_t \cdot D_0 \qquad (5)$$

where suffix t means that the pretreatment by bile salt aqueous solution was done. Therefore, the ratio of the membrane permeability coefficient before and after the pretreatment with bile salt solution can be shown in Eqn 6:

$$P_t^*/P^* = f_t/f \qquad (6)$$

Here, ϕ_t is assumed to be almost equal to ϕ, since the NaCl molecule is very small and it is not changed by the pretreatment. The value of P_t^*/P^*, therefore, represents the change in the membrane constant, f_t/f, which means the change in the membrane porosity because of the action

of bile salt on the membrane. In the permeability studies for dextran derivatives and insulin, the value of C* could not be obtained. The value of P after the pretreatment with bile salt aqueous solution, P_t, is shown in Eqn 7:

$$P_t = f_t \cdot \beta_t \cdot \phi_t \cdot D_0 \quad (7)$$

The ratio of P and P_t can then be shown as Eqn 8:

$$P_t/P = (f_t/f) \cdot (\beta_t \cdot \phi_t)/(\beta \cdot \phi) \quad (8)$$

The value of f_t/f was obtained from Eqn 6. The change in $\beta_t \cdot \phi_t/\beta \cdot \phi$ will be evaluated by measuring the permeability of dextran derivatives of various molecular weights.

For homopolymer, a linear relation is observed between the radius of gyration (R_H) of a molecule and the square root of the molecular weight (M_w) experimentally. The diffusion coefficient (D) of the spherical molecule is shown in Eqn 9:

$$D = (kT)/(6\pi\eta R_H) \quad (9)$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent. For the molecule that has the structure of a random coil, the relation between M_w and R_H is shown in Eqn 10:

$$M_w^{\frac{1}{3}} = (4\pi R_H^3)/3 \quad (10)$$

Judging from Eqns 9 and 10, the diffusion coefficient of homopolymer is proportional to the reciprocal of the square root of molecular weight.

Results and Discussion

It is well known that bile salts enhance bioavailability of polypeptides on nasal administration. Then, in this paper, the enhancement of the membrane permeability coefficient of polypeptides caused by the pretreatment with bile salts was measured in order to evaluate the effect of bile salt on nasal membrane.

Permeability for sodium chloride

The influence of bile salts on the nasal absorption of polypeptides was studied from the viewpoint of drug permeation. From the change in the permeability for NaCl by the pretreatment with bile salts, the change in the membrane constant was evaluated using Eqn 6.

Fig. 3 shows the relation between the permeation time and the concentration of NaCl in the receiver solution. An apparent linear relationship was observed. The membrane permeability coefficient of NaCl (P*) was approximately 6×10^{-7} cm^2/s and it was enhanced to approximately 11×10^{-7} cm^2/s by pretreatment with 10 mM DC saline solution.

The ratios of the membrane permeability coefficient of NaCl before pretreatment (P*) and after pretreatment (P_t*) by bile salts or EDTA are shown in Table 1. Membrane permeability for NaCl was not changed by the pretreatment procedure using saline solution ($P_t^*/P^* = 1.00$) which was done as a control. Also, EDTA enhanced the permeation of NaCl ($P_t^*/P^* = 1.64$). On the other hand, the values of P_t^*/P^* by pretreatment with C, DC, GC, GDC, TC and TDC were 1.24, 1.83, 1.66, 1.92, 1.87 and 1.87, respectively, showing the permeation enhancing effect of bile salts. The values of P_t^*/P^* reflect

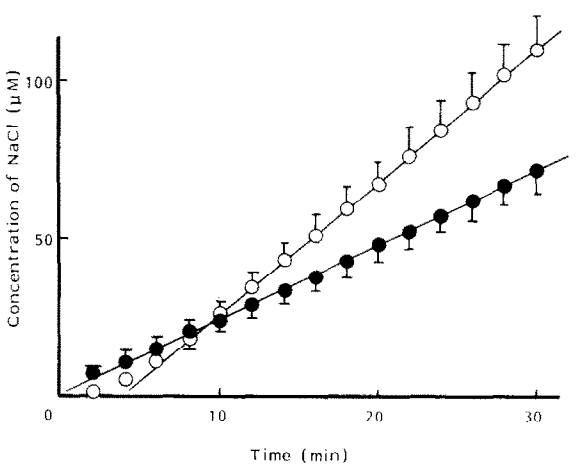

Fig. 3. Relationship between time and concentration of NaCl in the receiving cell at 36 °C. Data are given as mean ± SE ($n = 4$). ●, before pretreatment; ○, after pretreatment with 10 mM solution of DC.

TABLE 1

Influence of bile salts and EDTA on permeability coefficient in the nasal mucosa for NaCl in vitro

Agent added	$P^* \times 10^7$ (cm^2/s)	$P_t^* \times 10^7$ (cm^2/s)	P_t^*/P^*
Saline [a]	6.05 ± 1.83	6.21 ± 2.14	1.00 ± 0.04
C [a]	5.85 ± 1.30	7.25 ± 1.53	1.24 ± 0.04
DC [a]	6.01 ± 0.96	10.89 ± 1.51	$1.83 \pm 0.07^{\#}$
GC [b]	5.20 ± 2.20	8.44 ± 3.45	$1.66 \pm 0.05^{\#}$
GDC [b]	5.43 ± 1.55	10.27 ± 2.54	$1.92 \pm 0.16^{\#}$
TC [b]	5.75 ± 1.23	10.59 ± 2.47	$1.87 \pm 0.20^{\#}$
TDC [a]	5.39 ± 2.31	9.08 ± 2.95	$1.87 \pm 0.31^{\#}$
EDTA [c]	6.25 ± 1.56	9.34 ± 1.99	1.64 ± 0.15

Concentration of bile salts and EDTA was 10 mM. Data are given as mean ± SE.

[#] $P < 0.05$ (compared to the control (saline)).

[a] $n = 4$; [b] $n = 3$; [c] $n = 7$.

the change in the membrane porosity because of the effect of bile salts as shown in Eqn 6. DC and GDC showed a more remarkable effect on membrane permeability than C and GC did. DC and GDC are more lipophilic than C and GC, respectively, because of the lack of a hydroxyl group at the 7th position of the steroids. By employing a series of bile salts with subtle differences in number, position and orientation of their hydroxyl functions and alterations in side chain conjugation, it was suggested that the increase in permeability for sodium chloride correlates positively with increasing hydrophobicity of the bile salts.

Permeability for dextran derivatives

The permeabilities for dextran derivatives and insulin were measured to learn the pore size that bile salts create temporally in the membrane and the interaction between the membrane charge and the charged compound. As the absorption promoter GC was used because of its known ability as an effective absorption promoter for insulin and interferon (Maitani et al., 1990). The value of f_t/f by GC, which means the change in the membrane porosity, was already obtained from Table 1. Therefore, the value of $\beta_t \cdot \phi_t/\beta \cdot \phi$ in Eqn 8 will be evaluated by measuring the permeabilities for dextran derivatives which have neutral, positive and negative charges and various molecular weights.

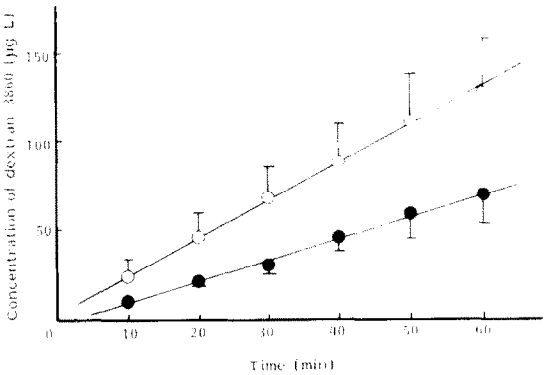

Fig. 4. Relationship between time and concentration of dextran-3860 in the receiving cell at 36 °C. Data are given as mean ± SE ($n = 4$). ●, before pretreatment; ○, after pretreatment with 10 mM solution of GC.

The relation between the permeation time and the concentration of DT in the receiver solution before and after the pretreatment with 10 mM solution of GC is shown in Fig. 4. An apparent linear relationship was observed. The values of P for DT before and after the pretreatment with 10 mM solution of GC, P and P_t were 1.59×10^{-7} cm^2/s and 2.70×10^{-7} cm^2/s, respectively.

Table 2 shows the values of P, P_t and P_t/P in the case of various molecular weights of DT, DE and DS. The values of P for dextran derivatives decreased with an increase of their molecular weights as the diffusion coefficients of these compounds with high molecular weights are low. The

TABLE 2

Influence of sodium glycocholate on permeability coefficient for dextran derivatives in vitro

Dextran derivatives	$P \times 10^8$ (cm^2/s)	$P_t \times 10^8$ (cm^2/s)	P_t/P
DT(mw. 3860)	15.91 ± 4.31	26.95 ± 5.60	1.69
DT(mw. 9000)	9.79 ± 4.33	11.60 ± 3.20	1.18
DT(mw. 17500)	7.92 ± 4.05	8.81 ± 1.16	1.11
DT(mw. 40500)	4.20 ± 1.85	7.27 ± 1.63	1.73
DE(mw. 6000)	20.03 ± 2.69	31.56 ± 12.01	1.58
DE(mw. 9000)	5.61 ± 1.30	19.16 ± 4.15	3.42
DS(mw. 5000)	8.67 ± 0.40	13.08 ± 5.28	1.51
DS(mw. 8000)	6.07 ± 2.93	4.65 ± 1.05	0.77

Concentration of sodium glycocholate was 10 mM. Data are given as mean ± SE ($n = 3$ or 4).

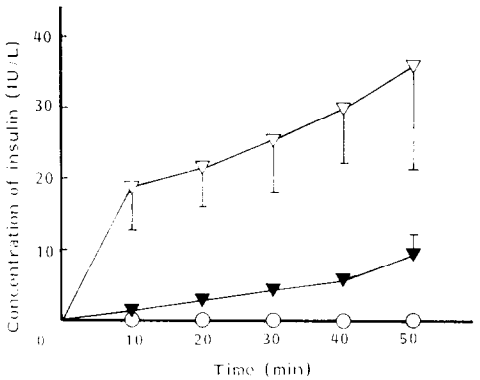

Fig. 5. Relationship between time and concentration of insulin in the receiving cell at 36 °C. Data are given as mean ± SE ($n = 3$). ▼, before pretreatment at pH 2.0; ▽, after pretreatment with 10 mM solution of GC at pH 2.0; ○, after pretreatment with 10 mM solution of GC at pH 5.4.

permeability was enhanced by the pretreatment with GC. The value of P for DE was higher than that for DT, and the value of P for DS was lower than that for DT which had the same molecular weight as DE and DS. The data for dextran derivatives suggest that the negatively charged compounds (DS) are repellent and the positively charged compounds (DE) give an access for drug diffusion into the membrane bearing a negative charge.

Permeability for insulin

As shown in Fig. 5, permeation of insulin was not observed at pH 5.4, even after the pretreatment with 10 mM solution of GC. The isoelectric point of insulin ranges from pH 5.3 to 5.35. Therefore, insulin in saline solution (pH 5.4) is not charged and forms a hexamer or octamer (Helmerhorst and Stokes, 1987), and its apparent molecular weight is 36000–48000.

On the other hand, insulin in saline solution (pH 2.0) is charged positively and forms a monomer, so that it is able to permeate the nasal mucosa (Klostermeyer and Humbel, 1966), which is negatively charged. The permeability coefficient of insulin was calculated using the slope. The slope was determined by five data points (at 10, 20, 30, 40 and 50 min) with the method of least squares, in order to obtain the average value of the amount of insulin permeated per unit time.

TABLE 3

Influence of sodium glycocholate on permeability coefficient for insulin in vitro

pH	$P \times 10^7$ (cm^2/s)	$P_t \times 10^7$ (cm^2/s)	P_t/P
5.4	0.0	0.0	–
2.0	9.95 ± 1.48	27.91 ± 1.92	2.87 ± 0.21

Concentration of sodium glycocholate was 10 mM. Data are given as mean ± SE ($n = 3$).

As shown in Table 3, the value of P for insulin at pH 2.0 was 9.95×10^{-7} cm^2/s, and the value of P for insulin after pretreatment with 10 mM solution of GC (P_t) was 2.79×10^{-6} cm^2/s. The permeability for insulin was enhanced 2.87 times by the pretreatment with GC.

Since dextran derivatives are homopolymer and have the structure of a random coil, the diffusion coefficient of compounds is generally proportional to the reciprocal of the square root of the molecular weight as shown in Eqns 9 and 10. Fig. 6 shows the correlation between P or P_t and molecular weights for dextran derivatives and insulin. The relation between P or P_t and the reciprocal of the square root of the molecular weights for dextran derivatives shows the linear-

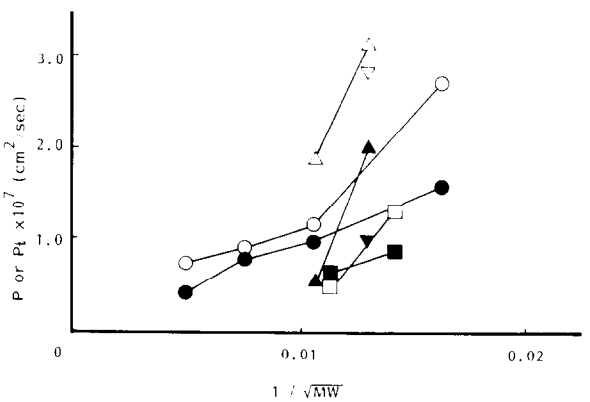

Fig. 6. Correlation between permeability coefficients P and P_t and molecular weights for dextran derivatives and insulin. ▼, insulin before pretreatment at pH 2.0; ▽, insulin after pretreatment at pH 2.0; ●, DT before pretreatment; ○, DT after pretreatment; ▲, DE before pretreatment; △, DE after pretreatment; ■, DS before pretreatment; □, DS after pretreatment. Pretreatments were done with 10 mM solution of GC.

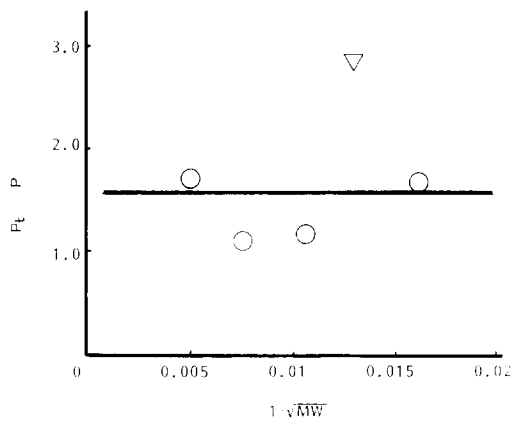

Fig. 7. Dependence of permeability coefficient ratio (P_t/P) on molecular weight. Pretreatments were done using 10 mM solution of GC. ▽, insulin at pH 2.0; ○, DT.

ity. It means that the values of P for DT, DE and DS decrease with increased molecular weight.

Fig. 7 shows the independence of P_t/P on the reciprocal of the square root of the molecular weight by pretreatment with 10 mM solution of GC. Comparing insulin with dextran of the same molecular weight, the value of P_t/P for insulin by pretreatment with GC was higher than that for hydrophilic dextran. This result suggests that insulin, which is hydrophobic, interacts with nasal mucosa pretreated by GC. Permeation is a complex phenomenon which combines the membrane constant, the partition coefficient and the diffusion coefficient, as shown in Eqn 2. Therefore, the interpretation of the partition coefficient is normally difficult unless the three contributions can be separated. However, the change in the three factors by pretreatment with GC (f_t/f, ϕ_t/ϕ, β_t/β) is the indicator governing nasal membrane transport. The value of f_t/f is constant and is assumed to be equal to 1.66 as in Table 1, because GC is used as an absorption promoter for insulin and dextran. Since P_t/P for dextran is about 1.66, $(\phi_t/\phi)\cdot(\beta_t/\beta)$ is almost equal to 1.0, indicating that the interaction between the dextran and the membrane has not been changed by the pretreatment. On the other hand, the value of P_t/P for insulin is 2.87, which gives 1.73 for $(\phi_t/\phi)\cdot(\beta_t/\beta)$. The value of (ϕ_t/ϕ) is almost the same for DT (mw. 9000) and insulin (mw. about 6000) since their molecular weights are almost the same. The value of (ϕ_t/ϕ) is, therefore, assumed to be 1.0, and (β_t/β) for insulin is 1.73. It means that the value of the partition coefficient of insulin between the solution and the membrane surface, β_t, was increased by pretreatment with GC.

Conclusion

From the change in the membrane permeability coefficient of sodium chloride, it is known that the increase in permeability for sodium chloride correlates positively with increasing hydrophobicity of bile salts. It seems that the dihydroxy bile salts are more effective as absorption promoters in the nasal mucosa. It is suggested that higher permeability should be expected if the drug is positively charged, as rabbit nasal mucosa is a negatively charged membrane. It seemed that bile salts enhanced the permeability for water-soluble compounds which have high molecular weights (mw. 40500) since the temporal pores in the membrane which bile salts create are not small. The permeability for dextran derivatives in nasal mucosa increased 1.66 times because of the pretreatment with sodium glycocholate. This increase is caused by an increase in the membrane porosity, because the value 1.66 was equal to the value of (P_t/P) for NaCl. The (P_t/P) value for insulin was 2.87, which is considered to be the product of 1.66 for the increase in membrane porosity multiplied by 1.73 for the increase in the partition coefficient of insulin in the nasal mucosa by the pretreatment with sodium glycocholate.

Acknowledgement

The authors are grateful to Mr Hisao Ohtsuki for assistance with the experimental work.

References

Belder A.N. and Granath, K., Preparation and properties of fluorescein-labelled dextrans. *Carbohydr. Res.*, 30 (1973) 375–378.

Chien, Y.W., *Transnasal Systemic Medications*. Elsevier, Amsterdam, 1985.

Gordon, G.S., Moses, A.C., Silver, R.D., Flier, J.S. and Carey, M.C., Nasal absorption of insulin: enhancement by hydrophobic bile salts. *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 7419–7423.

Helmerhorst, E. and Stokes, G.B., Self-association of insulin: its pH dependence and effect of plasma. *Diabetes*, 36 (1987) 261–264.

Hirai, S., Yashiki, T. and Mima, H., Effect of surfactants on the nasal absorption of insulin in rats. *J. Pharm. Sci.*, 9 (1981) 165–172.

Klostermeyer, H. and Humbel, R.E., The chemistry and biochemistry of insulin. *Angew. Chem., Int. Edition*, 5 (1966) 807–822.

Kobatake, Y., Takeguchi, T., Toyoshima, Y. and Fujita, Y., Studies of membrane phenomena. 1: membrane potential. *J. Phys. Chem.*, 69 (1965) 3981–3988.

Maitani, Y., Igawa, T., Machida, Y. and Nagai, T., Plasma levels following intranasal and intravenous administration of human interferon-β to rabbits. *Drug Des. Delivery*, 4 (1988) 109–119.

Maitani, Y., Machida, Y. and Nagai, T., Influence of molecular weight and charge on the nasal absorption of dextran and DEAE-dextran in rabbits. *Int. J. Pharm.*, 49 (1989) 23–27.

Maitani, Y., Uchida, N., Taniguchi, M., Yamazaki, S., Hara, M., Takayama, K., Machida, Y. and Nagai, T., The inactivation process of fibroblast interferon-β in the preparation stage and in rabbit nasal absorption of mixed and freeze-dried powder dosage forms. *Int. J. Pharm.*, 64 (1990) 139–146.

Maitani, Y., Uchida, N., Nakagaki, N. and Nagai, T., *Int. J. Pharm.*, 1991.

McKernan, W.M. and Ricketts, C.R., A basic derivative of dextran and its interaction with serum albumin. *Biochem. J.*, 76 (1960) 117–120.

Nakagaki, M., Koga, N. and Iwata, S., Bio-physico chemical studies on phenoxazone compounds, 1: diffusion constant and degree of association. *Yakugaku Zasshi*, 82 (1962) 1138–1141.

Wheatley, M.A., Dent, J., Wheeldon, E.B. and Smith, P.L., Nasal drug delivery: an in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. *J. Controlled Release*, 8 (1988) 167–177.