ELSEVIER

International Journal of Pharmaceutics 146 (1997) 11–19

international journal of pharmaceutics

In vitro nasal transport across rabbit mucosa: effect of oxygen bubbling, pH and hypertonic pressure on permeability of lucifer yellow, diazepam and 17 β-estradiol

Y. Maitani*, K. Ishigaki, K. Takayama, T. Nagai

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

Received 18 April 1996; revised 29 June 1996; accepted 4 September 1996

Abstract

The effect of pH, oxygen bubbling and hypertonic osmolarity in excised rabbit nasal mucosa on permeation of drugs was examined. For preincubation we used: (i) pH 5.2 saline, pH 7.4 saline and Krebs-Ringer solution (KR); (ii) the absence and presence of oxygen bubbling; and (iii) hypertonic KR. The control was put in KR with oxygen bubbling without preincubation. The permeation of lucifer yellow (LY; MW 521) as a hydrophilic drug and of diazepam (DP; MW 285) as a lipophilic drug and the amount of protein released from tissue were measured after preincubation. Also the enzyme activity in the nasal mucosa was examined by measuring the permeation of 17 β-estradiol (E2; MW 272) and estrone (El; MW 270) that was produced from E2 by 17 β-hydroxy-steroid dehydrogenase (17 β-HSD). The low pH increased the permeation of drugs, especially LY. The activity of 17 β-HSD appears to decrease by a low pH more than by bubbling. The protein from the mucosa after preincubation was released in the donor and receiver cells without bubbling irrespective of pH. High osmotic pressure in donor KR solution with bubbling also released more protein in the donor cell compared with a change in pH to 5.2 and 7.4 without bubbling. The results of the released protein amount are in agreement with those of the permeability study, suggesting a morphological change. The low pH increased the permeation of drug, and decreased the enzyme activity in the nasal mucosa without increasing the release of protein from the nasal mucosa compared with the release at pH 7.4. This finding can be applied to nasal absorption of drugs.

Keywords: Nasal transport; Hypertonic pressure; Oxygen bubbling; Rabbit mucosa; Diazepam; 17 β-Estradiol

1. Introduction

At present there is an increasing interest for systemic application of drugs via the nasal cavity. The nasal route is effective and acceptable for

* Corresponding author.

0378-5173/97/$17.00
PII S0378-5173(96)04750-3

drugs. Nasal administration offers several advantages, including rich vascularity, avoidance of first-pass metabolism, and absence of an unfavorable luminal environment like that found in the intestine.

We have studied the nasal absorption of peptide drugs such as insulin (Uchida et al., 1991; Maitani et al., 1992) and erythropoietin (Shimoda et al., 1995) in vivo and in vitro. In these cases, we found an increase of absorption at a low medium pH. Also, it is reported that the pharmacological availabilities after nasal administration of insulin (Hirai et al., 1981), secretin (Ohwaki et al., 1987), vasopressin (Morimoto et al., 1991) and calcitonin (Morimoto et al., 1995) at low pH were increased in rats in vivo.

The rate and extent of nasal drug absorption may depend on a number of physicochemical factors, i.e. partition coefficient and molecular weight of drug, pH and osmolarity of bathing solution and oxygen bubbling. The low pH without bubbling and osmolarity may affect the nasal mucosa and then may increase the permeation of drugs.

Various methods have been used to test the viability and integrity of mucosa. Traditionally, the membrane potential (Maitani et al., 1991), electric resistance (Hidalgo et al., 1993), the short-circuit current (Bechgaard et al., 1992; Reardon et al., 1993) and activity of enzyme (Pujara et al., 1995) in membrane are used. In these in vitro experiments, usually the tissue was equilibrated for about 1 h prior to beginning each permeability study. Therefore, to determine the early stage of change in the nasal mucosa, the permeation of drugs after various preincubation was measured. Also, the effect of enzyme activity on the permeation and the amount of protein released were measured to obtain additional information about the intracellular damage in the nasal mucosa. The total protein release data although not very specific in the type of damage, provide a general indication about the extent of the integrity.

The objective of this study was to investigate the effect of pH, oxygen bubbling and hypertonic osmolarity in excised rabbit nasal mucosa on the permeation of drugs. Various pHs in bathing solution with and without bubbling of gas using 95% O_2/5% CO_2 (oxygen) and hypertonic solution were used for preincubation and then the amount of protein released from the tissue was measured. The permeation of lucifer yellow (LY; MW 521) for hydrophilic drugs and of diazepam (DP; MW 285) for lipophilic drugs after preincubation was measured. Also, the enzyme activity in the nasal mucosa as skin was examined by measuring the permeation of 17 β-estradiol (E2; MW 272) and estrone (El; MW 270) that was produced from E2 by 17 β-hydroxy-steroid dehydrogenase (17 β-HSD) (Liu et al., 1991).

2. Experimental

2.1. Materials

LY (potassium salt) was purchased from Sigma. DP was purchased from Wako Pure Chemical (Japan). E2 and El were purchased from Tokyo Kasei Kogyo (Tokyo). All other chemicals used were of reagent grade.

2.2. Permeation studies

Male Japanese white rabbits (2.5–3.0 kg, Saitama Experimental Animal Supply, Japan) were killed with an overdose of sodium pentobarbital through the marginal ear vein. The nasal mucosa used in these experiments was obtained from the anterior orbits of the junction of the nasal bone with the dorsal pariental cartilage, as previously described (Maitani et al., 1992). After a piece of the nasal mucosa was immediately placed in oxygenated Krebs-Ringer solution (KR), it was mounted as a flat sheet on a circular window with a 0.503 cm^2 area. After mounting the tissue in the diffusion cell with the mucosal side facing the donor cell, the temperature was maintained at 36 ± 0.5°C during the course of an experiment.

For the 1 h preincubation, we used: (i) pH 5.2 saline solution, pH 7.4 saline solution adjusted by using sodium hydroxide solution and KR (ii) with and without bubbling of oxygen and (iii) hypertonic KR. The preincubation was done as follows, the donor and receiver cells were filled with a solution of saline solution (pH 5.2, 7.4) and KR

with and without bubbling of oxygen. The hypertonic condition in donor solution was adjusted by sodium chloride in KR at 600 mOsm with bubbling, whereas the receiver solution was KR. As a control the same procedure was done in KR with oxygen bubbling without preincubation. The bathing saline solution was adjusted to the appropriate pH using hydrochloride. At the end of the preincubation period, chambers were drained, tissue was subsequently washed and refilled with a 6 ml of drug and KR solution in the mucosal bath and serosal bath, respectively. LY solution (0.02%) and DP and E2 suspension were used as the donor solution, respectively. Aliquots were withdrawn with a micropipette from the receiver cell and donor cell for E1 at every 1 h and replaced with warm buffer over a 5 h period with oxygen bubbling. Then the thickness was measured by a thickness gauge with an accuracy of ± 0.001 mm. Samples were analyzed by HPLC for LY, DP, E2 and E1.

The equipment used included: Shimadzu pump; SPD-6AV UV spectrophotometer (Shimadzu, Kyoto, Japan); C-R6A chromatopack; a YMC C8 column for DP and a YMC C18 column for E2 and E1 (Yamamura, Tokyo). The mobile phases were a 30:70 (v/v) mixture of 0.5 mol/l acetic acid aqueous solution and methanol, containing 5 mmol/l 1-pentanesulfonic acid monohydrate sodium salt for DP, and a (65:35 (v/v) mixture of water and acetonitile): diethylether (90:10) for E2. DP and E2 were detected by their UV absorbance at 230 (Mannucci et al., 1993) and 280 nm (Liu et al., 1990), respectively. Fluorescence of LY samples was measured at excitation 428 and emission 540 nm (Reardon et al., 1993).

From the plots of the concentration of permeant appearing in the receiver cell with time, the permeability coefficients (K) were calculated. The flux of mass from the donor cell is assumed to be equal to the appearance rate of mass in the receiver.

$$J = AKC = V\,(dCr/dt) \tag{1}$$

where J is the flux, A is the area of a circular window, C and Cr are the drug concentrations in the donor and receiver cells, respectively, V is the volume of the donor and receiver cells (6 ml) and t is time. Experimentally, C does not appreciably change during the experimental period; therefore, sink conditions in the receiver cell essentially prevail. Consequently, K is determined by using Eq. (1).

2.3. Determination of diffusion coefficient and enzyme rate constant

Diffusion coefficients and enzyme rate constants are calculated from the analysis of experiments on the simultaneous transport and metabolism of E2 in mucosa using the model approach reported by Liu et al., (1990). The membrane is depicted by the diffusion model in Fig. 1. The changes in concentrations of drug and metabolite with the depth in the membrane, thickness (x for $0 \leqslant x \leqslant h$, $h = 346.2 \pm 26.2$ μm) are described. In the steady state, we may write

$$D_{E2}\frac{d^2C_{E2}}{dx^2} - kC_{E2} = 0 \tag{2}$$

$$D_{E1}\frac{d^2C_{E1}}{dx^2} + kC_{E2} = 0 \tag{3}$$

where D is the diffusion coefficients, k is the apparent first-order enzyme rate constant and C is the concentrations in receiver cells. The subscripts refer to E2 and E1.

When the critical condition is $x = h$, $C_{E2} = 0$ and $C_{E1} = 0$, and when $x = 0$, $C_{E2} = 1$ and $C_{E1} = 0$, Eqs. (2) and (3) were rewritten as follows:

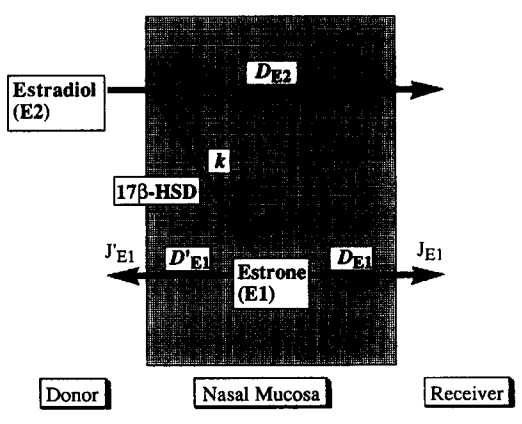

Fig. 1. Diffusion model of E2 and E1 in nasal mucosa.

Table 1
Influence of preincubation on permeability coefficients (K) of LY across nasal mucosa

Preincubation solution	pH	Oxygen[a]	$K \times 10^6$ (cm/s)	K/K_0
Control[b]			0.633 ± 0.052 (K_0)	1
Saline	5.2	+	0.948 ± 0.048	1.497 ± 0.145*
		−	1.145 ± 0.081	1.809 ± 0.196**
	7.4	+	0.731 ± 0.086	1.155 ± 0.166
		−	1.111 ± 0.040	1.755 ± 0.157**
KR	7.4	+	0.745 ± 0.061	1.177 ± 0.137
		−	1.034 ± 0.095	1.633 ± 0.201*
Hypertonic[c]	7.4	+	1.196 ± 0.140	1.889 ± 0.270**

Data are given as mean ± S.E. ($n = 3 \sim 4$).
[a] Oxygen bubbling (+), no bubbling (−).
[b] In KR solution at pH 7.4 with oxygen bubbling without preincubation; isotonic.
[c] Hypertonic; 600 mOsm in KR solution adjusted by NaCl at pH 7.4 with bubbling.
* $P<0.05$, ** $P<0.01$ compared with control (K_0).

$$\frac{J_{E2}}{C_{E2}} = aD_{E2}\,\mathrm{cosech}(ah) = 0 \quad (4)$$

$$\frac{J_{E1}}{C_{E2}} = aD_{E2}\sinh(ah) + \frac{D_{E2}}{h} - aD_{E2}\cosh(ah)\cot(ah) = 0 \quad (5)$$

$$a = \sqrt{\frac{k}{D_{E2}}}$$

here, with boundary concentrations, the calculated fluxes were fitted to the experimental data by computer. Two parameters, k and D_{E2}, were adjusted in bestfitting the data from each set of experiments to model.

2.4. Determination of partition of drugs

KR and isopropyl myristate phases were equilibrated with each other prior to the commencement of the partitioning experiment. Isopropyl myristate (3 ml) was added to KR (3 ml) containing each drug in a test tube. The partition coefficients were obtained by measuring the drug concentration in the aqueous and/or oil phases after incubation (3–7 days) at 36°C.

2.5. Protein content assay

The amount of protein in the cells after preincubation was determined using a BCA protein assay reagent from Pierce (Rockford, IL) with bovine serum albumin as the standard.

2.6. Statistics

The results are presented as means ± S.D. or S.E. Statistical analysis was performed with Student's paired or unpaired t test as appropriate. A value of P less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Effect of pH and bubbling on permeation of LY or DP

The partition coefficients (log P) of LY, DP and E2 were −1.33, 2.28 and 2.68, respectively. LY was used to produce the passive permeability (Reardon et al., 1993). Table 1 shows the influence of preincubation on K of LY in saline at pH 5.2 and 7.4, in KR at pH 7.4 and hypertonic pressure for bathing solutions. K was normalized with that of control (K_0). When the tissue was preincubated with bubbling, K of LY did not show any difference among KR, saline at pH 7.4 and control, i.e. no preincubation in KR ($P > 0.05$). The K/K_0 value of LY without bubbling increased significantly. Even with bubbling pH 5.2 increased the K of LY significantly compared with the control ($P < 0.05$).

Table 2 shows the influence of preincubation on K of DP in various bathing solutions. When the

Table 2
Influence of preincubation on permeability coefficients (K) of diazepam across nasal mucosa

Preincubation solution	pH	Oxygen[a]	$K \times 10^6$ (cm/s)	K/K_0
Control[b]			7.09 ± 1.376 (K_0)	1
Saline	5.2	+	8.62 ± 1.000	1.216 ± 0.275
		−	12.13 ± 1.580	1.711 ± 0.400**
	7.4	+	6.78 ± 0.956	0.956 ± 0.229
		−	9.91 ± 0.982	1.398 ± 0.305*
KR	7.4	+	6.55 ± 0.621	0.924 ± 0.200
		−	10.49 ± 0.621	1.480 ± 0.300*
Hypertonic[c]	7.4	+	10.75 ± 1.739	1.516 ± 0.383*

Data are given as mean ± S.D. ($n = 3 \sim 4$).
[a] Oxygen bubbling (+), no bubbling (−).
[b] In KR solution at pH 7.4 with oxygen bubbling without preincubation; isotonic.
[c] Hypertonic; 600 mOsm in KR solution adjusted by NaCl at pH 7.4 with bubbling.
* $P<0.05$, ** $P<0.01$ compared with control (K_0).

tissue was preincubated with bubbling, K of DP did not show any difference among KR, saline at pH 7.4 and control. Saline at pH 7.4 and KR with bubbling did not appear to affect the mucosa since K of DP at these conditions were similar to the control. The K values of DP without bubbling increased significantly compared with the control. The saline at pH 5.2 increased the K of DP even with bubbling.

The K/K_0 of LY and DP was measured in various media with and without bubbling. LY permeation appears to be more affected by saline at pH 5.2 with bubbling and by saline at pH 7.4 without bubbling compared with DP. Also bubbling affected the increase of permeation of both drugs greater than pH. Oxygen bubbling was suggested to be the most important factor to maintain integrity of the excised nasal mucosa.

3.2. Effect of pH and bubbling on enzyme in permeation of E2

The permeation of E2 was measured after preincubation since it is suitable to examine enzyme activity in the mucosa. El was produced by 17 β-HSD from E2 in the mucosa during the permeation of E2. Various pH and bubbling in preincubation may affect enzymes in the mucosa and therefore change permeation of E2 and E1.

Table 3 shows the forward flux of E2 and El to receiver (J_{E2}, J_{E1}) and the back flux of El (J'_{E1}) to donor cell after preincubation. They are normalized by the solubility of E2, C_{E2} (2.478 μg/ml). The flux of E2 in various media cannot be compared simply with the control since E2 changes to El by 17 β-HSD. PH, medium and bubbling in preincubation affected J_{E2}, J_{EI} and J'_{EI} since the activity of 17 β-HSD was influenced by preincubation. Saline at pH 5.2 without bubbling increased J_{E2} but KR did not. J_{EI} decreased after preincubation, but J'_{E1} in KR without bubbling was not changed compared with the control.

3.3. Effect of osmolarity

Tables 1 and 2 show the influence of osmotic pressure on the mucosal side in preincubation on K/K_0 of drugs across nasal mucosa with bubbling. The KR isotonic solution did not increase the K value compared with the control. K/K_0 of both drugs significantly increased in a hypertonic solution compared with an isotonic solution.

3.4. Released protein after preincubation

Fig. 2 shows the influence of pH and bubbling in preincubation on the release of protein from the nasal mucosa. Protein was released even in the KR at pH 7.4. Under bubbling, about 30–50 μg/ml protein was released in both side cells. Without bubbling, the amount of protein released was two times greater than that with bubbling,

Table 3
Simultaneous determination of diffusion coefficient of 17 β-estradiol (D_{E2}) and rate constant for bioconversion from 17β-estradiol to estrone (k)

Preincubation				Flux/C^a_{E2} ($\times 10^7$, cm/s)			Parameters	
Solution	pH	Oxygen[b]		J_{E2}/C^c_{E2}	J_{E1}/C^c_{E2}	J'_{E1}/C^d_{E2}	$k \times 10^5$ (s^{-1})	$D_{E2} \times 10^7$ (cm^2/s)
Control			Observed	79.9 ± 12.8	12.46 ± 4.80	5.75 ± 1.90	—	—
			Calculated[e]	75.3	9.67	—	18.2	2.94
Saline	5.2	−	Observed	148.3 ± 17.4	4.43 ± 0.78	2.42 ± 0.64	—	—
			Calculated	144.1	4.11	—	7.3	5.13
KR	7.4	−	Observed	73.0 ± 6.3	2.88 ± 0.79	5.19 ± 1.70	—	—
			Calculated	75.4	2.46	—	4.4	2.69
	7.4	+	Observed	63.3 ± 4.3	2.66 ± 0.23	2.82 ± 0.23	—	—
			Calculated	61.7	2.24	—	4.0	2.21

[a] The experimental normalized fluxes are expressed as the mean ± S.D. ($n = 3 \sim 4$).
[b] Oxygen bubbling (+), no bubbling (−).
[c] J_{E2} and J_{E1} represent the forward flux of E2 and E1 to receiver, respectively.
[d] J'_{E1} represents the back flux of E1 to donor.
[e] Theoretical fluxes were calculated using iteration parameters, Eqs. (4) and (5), and mean values of the data.

and was almost same one under various pH and medium.

Fig. 3 shows the influence of osmotic pressure in preincubation on the release of protein from nasal mucosa. A hypertonic donor solution increased the release of protein from the mucosa to donor cell compared with an isotonic one. The released protein amount in the donor cell appeared to be higher than that by changing pH and medium (Fig. 2).

4. Discussion

4.1. pH and bubbling

The flux of peptide drug at a low pH increased in vitro (Maitani et al., 1992) and in vivo (Shimoda et al., 1995). In vitro, the microclimate pH gradient may be less than that encountered in vivo due to the more efficient mixing. This possibility was investigated by reduction of the pH of

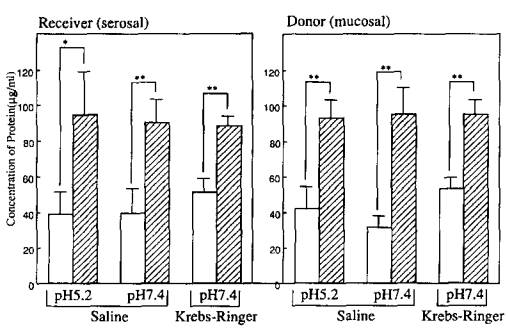

Fig. 2. Influence of preincubation in various solutions for 1 h on the release of protein from the nasal mucosa to donor and receiver cells (6 ml). Data are given as mean ± S.D. ($n = 3$); □, with bubbling; ■, without bubbling. * $P < 0.05$, ** $P < 0.01$ compared with no bubbling.

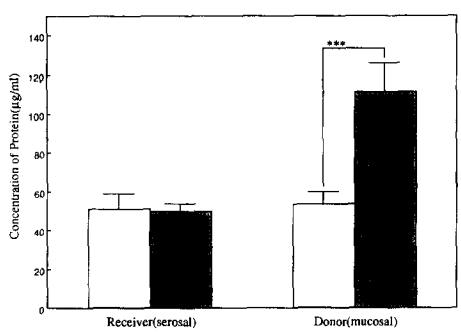

Fig. 3. Influence of osmotic pressure on the mucosal side in preincubation for 1 h on release of protein from nasal mucosa to donor and receiver cells (6 ml). Data are given as mean ± S.D. ($n = 3$). ■, Hypertonic; 600 mOsm in KR solution adjusted by NaCl at pH 7.4 with bubbling. □, Isotonic; KR solution at pH 7.4 with bubbling (Tables 1 and 2). *** $P < 0.001$ compared with isotonic.

the mucosal bathing solution from pH 7.4 to 5.2. The change of pH in the bathing solution affects the drug ionization to alter its permeability characteristics. However, LY is not dissociated and is already known to be transported passively as is mannitol (Reardon et al., 1993). Therefore, only a change of pH may affect the mucosa. Even with bubbling, at pH 5.2 the flux of LY was significantly higher than that in the control. The absence of bubbling increased the K of drugs more than the low pH.

Hidalgo et al. (1993) reported that integrity of the intestinal preparations was assessed through the measurements of the transepithelial conductance, and fluxes of mannitol increased by reducing the pH from 7.4 to 5 in the mucosal bathing solution with bubbling, not changing the electrical properties. Our finding on LY is consistent with that on mannitol.

Table 3 shows the fluxes of E2 and El, and D_{E2} and k values for bioconversion from E2 to E1 after various preincubation conditions. D_{E2} was determined through the use of Eqs. (4) and (5), which simultaneously calculated k and D. Calculated D_{E2} gave similar values except for saline at pH 5.2. The pH 5.2 without bubbling affected the mucosa and increased the J_{E2}. Bubbling at pH 7.4 appears to maintain integrity of the excised mucosa for 6 h, the total duration of preincubation and permeation study since the J_{E2} did not increase compared with the control. This corresponds well to that of DP showing almost the same partition coefficient as E2 (Table 2). However, the k value decreased after preincubation with bubbling even at pH 7.4 compared with the control. This finding suggests that the k value decreased since the activity of 17 β-HSD decreased after preincubation, and that the integrity of the mucosa after preincubation cannot be estimated from the J_{E2}. Pujara et al. (1995) used lactate dehydrogenase and 5′-nucleotidase, and Marttin et al. (1995) used acid phosphatase released from the nasal mucosa for marker enzymes for the viability and integrity of nasal mucosa. The k value in the control may be the marker measuring permeation of drugs without measuring the enzyme activity separately. The J'_{E1} in KR without bubbling was higher than the J_{E1} with bubbling in KR and in saline at pH 5.2. This might be due to the fact that 17 β-HSD is distributed near the surface of the tissue and E1 can penetrate to the donor more easily than to the receiver.

The low pH increased the permeation of drugs and especially LY, hydrophilic one. One of the reasons might be the increase in the hydration of mucosa with a decrease of pH. The permeation of hydrophilic drug may be more affected by hydration than that of a lipophilic drug since the pathways of hydrophilic and lipophilic drugs may be different (Behl and Barrett, 1981). The hydration-related permeability changes in tissues could be due to some modification of the hydrophilic pathway in tissue, and the exact mechanism for it is unknown. Another reason may be the reduction in the biological integrity in the mucosa. The activity of 17 β-HSD, which is responsible for the condition of the mucosa, appears to be affected by pH more than by oxygen bubbling (Table 3).

Pujara et al. (1995) have studied the nasal epithelial cell integrity from the release of cytosolic and membrane-bound enzymes in situ nasal perfusion technique changing pH from 2 to 12 and osmolarity. Their findings indicate that solutions with a pH range of 3–10 and hypertonic and isotonic sodium chloride solution caused minimal release of the enzyme. These findings were not coincident with ours. The enzymes may have specificity with the condition.

However, the released protein from nasal mucosa after preincubation with bubbling was not different compared with that in KR at pH 7.4, i.e. control (Fig. 2). The release of protein from the mucosa was increased without bubbling irrespective of pH. Bubbling appears to keep integrity of the mucosa and this finding is consistent with the result of the permeability analysis (Tables 1 and 2). A similar amount of protein was released from the mucosa to donor and receiver cells. Control in KR at pH 7.4 released about 2% of total protein in the nasal mucosa after 1 h preincubation when the total protein in the mucosa was estimated using the permeated areas (0.503 cm^2), the thickness (346.2 ± 26.2 μm) and the density of the nasal mucosa (0.855 ± 0.008 g/ml, $n = 4$, mean $\pm$ S.D.).

4.2. High osmotic pressure

High osmotic pressure in the donor cell with bubbling also affects the mucosa as described in the change of the permeability (Tables 1 and 2). This corresponds well to the effects of secretin (Ohwaki et al., 1987) and erythropoietin in rats (Shimoda et al., 1995). High osmolarity of sodium chloride released more protein from the mucosa in donor cells than in receiver cells. Therefore, high osmolarity may have affected the epithelial cells to release the protein to donor cell and increased the permeation of drugs.

From the released protein, no bubbling and high osmotic pressure affected the mucosa to extract the protein from it and the former may act on the whole tissue whereas the latter on the surface since the latter released protein in donor cell but the former in both cells (Fig. 3). The low pH with bubbling (about 40 μg/ml released protein) may not affect the mucosa more than that without bubbling in KR (92.5 μg/ml) and high osmolarity (111.8 μg/ml) since a similar amount of protein was extracted as at pH 7.4 (Fig. 2). This finding corresponds to that without bubbling and high osmolarity in KR increased the permeation (the former for K/K_0 of LY and DP, 1.63, 1.48, the latter for K/K_0 of DP and LY, 1.87, 1.51, respectively) more than pH 5.2 with bubbling (for K/K_0 of LY and DP, 1.50, 1.22, respectively) (Tables 1 and 2). The mechanism involved in the increase in permeability may be different with pH, bubbling and high osmotic pressure.

The transepithelial conductance and short-circuit current were often used to monitor the viability and integrity of tissue. They achieved stable values after approximately 3 h in vitro (Reardon et al., 1993), but the mucosa was exposed to change during this period. Bechgaard et al. (1992) reported that the isolated nasal mucosa is viable for more than 10–12 h in the Ussing chamber in bicarbonate Ringer solution with bubbling gas (95% O_2/5% CO_2). However, our finding suggests that the permeation characteristic is altered by the condition of preincubation for 1 hr. The condition of equibriated state before measuring membrane potential is important to maintain the integrity of the tissue.

We reported the influence of a 16-h incubation in potassium chloride on the membrane potential (Maitani et al., 1991). The membrane potential of the nasal mucosa is neutralized suggesting that the charged component of the membrane is dissolved into the solution. The present findings also are in agreement. The release of protein, without bubbling, may affect the mucosal and serosal side of the nasal mucosa whereas the high osmolarity affects the mucosal side. The low pH appears to affect the mucosa and to decrease enzyme activity.

5. Conclusion

The low pH increased the permeability of the hydrophilic drug, LY, more through the mucosa compared with hydrophobic drug, DP, even with bubbling. This may be due to the difference in the permeation mechanism of the hydrophilic drug from the hydrophobic drug. The low pH and no oxygen inhibit the biochemical conversion of E2 to E1 since the 17 β-HSD activity decreased. The low pH acts as a transport enhancer for E2 across the nasal mucosa and acts as an inhibitor of the enzyme in the mucosa without irreversible structure change of the mucosa, i.e. extracting protein.

The present findings will aid the pharmaceutical scientist to estimate correctly the permeation of nasal absorption of drugs from in vitro permeation study.

Acknowledgements

The authors are grateful to Mr K. Shimizu, Mr K. Tachihara and Ms S. Kobayashi for assistance in experimental work.

References

Bechgaard, E., Gizurarson, S., Jørgensen, S. and Larsen, R., The viability of isolated rabbit nasal mucosa in the ussing chamber, and the permeability of insulin across the membrane. *Int. J. Pharm.*, 87 (1992) 125–132.

Behl, C.R. and Barrett, M., Hydration and percutaneous absorption II: Influence of hydration on water and alkanol permeation through swiss mouse skin; comparison with hairless mouse. *J. Pharm. Sci.*, 70 (1981) 1212–1214.

Hidalgo, I.J., Ryan, F.M., Marks, G.J. and Smith, P.L., pH-dependent transepithelial transport of cephlexin in rabbit intestinal mucosa. *Int. J. Pharm.*, 98 (1993) 83–92.

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

Liu, P., Higuchi, W.I., Ghanem, A.H., Bergstrom, T.K. and Good, W.R., Quantitation of simultaneous diffusion and metabolism of β-estradiol in hairless mouse skin: enzyme distribution and intrinsic diffusion/metabolism parameters. *Int. J. Pharm.*, 64 (1990) 7–25.

Liu, P., Higuchi, W.I., Song, W.-Q., Bergstrom, T.K. and Good, W.R., Quantitative evaluation of ethanol effects on diffusion and metabolism of β-estradiol in hairless mouse skin. *Pharm. Res.*, 8 (1991) 865–872.

Maitani, Y., Uchida, N., Nakagaki, M. and Nagai, T., Effect of bile salts on the nasal mucosa: Membrane potential measurement. *Int. J. Pharm.*, 69 (1991) 21–27.

Maitani, Y., Asano, S., Takahashi, S., Nakagaki, M. and Nagai, T., Permeability of insulin entrapped in liposome through the nasal mucosa of rabbits. *Chem. Pharm. Bull.*, 40 (1992) 1569–1572.

Mannucci, C., Bertini, J. and Cocchini, A., High-performance liquid chromatographic method for assay of otilonium bromide, diazepam, and related compounds in finished pharmaceutical forms. *J. Pharm. Sci.*, 82 (1993) 367–370.

Marttin, E., Verhoef, J.C., Romeijn, S.G. and Merkus, W.H.M., Effects of absorption enhancers on rat nasal epithelium in vivo: release of marker compounds in the nasal cavity. *Pharm. Res.*, 12 (1995) 1151–1157.

Morimoto, K., Yamaguchi, H., Iwakura, Y., Morisaka, K., Ohshi, Y. and Nakai, Y., Effect of viscous hyaluronate-sodium solutions on the nasal absorption of vasopressin and an analogue. *Pharm. Res.*, 8 (1991) 471–474.

Morimoto, K., Miyazaki, K. and Kakemi, K., Effect of proteolytic enzyme inhibitors on nasal absorption of salmon calcitonin in rats. *Int. J. Pharm.*, 113 (1995) 1–8.

Ohwaki, T., Ando, H., Kakimoto, F. Uesugi, K., Watanabe, S., Miyake, Y. and Kayano, M., Effects of dose, pH, and osmolarity on nasal absorption of secretin in rats II: histological aspects of the nasal mucosa in relation to the absorption variation due to the effects of pH and osmolarity. *J. Pharm. Sci.*, 76 (1987) 695–698.

Pujara, C.P.Z., Duncan, M.R. and Mitra, A.K., Effects of formulation variables on nasal epithelial cell integrity: Biochemical evaluations. *Int. J. Pharm.*, 114 (1995) 197–203.

Reardon, P.M., Gochoco, C.H., Audus, K.L., Wilson, G. and Smith, P.L., In vitro nasal transport across ovine mucosa: effects of ammonium glycyrrhizinate on electrical properties and permeability of growth hormone releasing peptide, mannitol, and lucifer yellow. *Pharm. Res.*, 10 (1993) 553–561.

Shimoda, N., Maitani, Y., Machida, Y. and Nagai, T., Effects of dose, pH and osmolarity on intranasal absorption of recombinant human erythropoietin in rats. *Biol. Pharm. Bull.*, 18 (1995) 734–739.

Uchida, N., Maitani, Y., Machida, Y., Nakagaki, M. and Nagai, T., Influence of bile sale on the permeability of insulin through the nasal mucosa of rabbits in comparison with dextran derivatives. *Int. J. Pharm.*, 74 (1991) 95–103.