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Effect of bile salts on the nasal mucosa: Membrane potential measurement

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Summary

The membrane potential of rabbit nasal mucosa was measured in vitro to investigate the electrical properties of the tissue. The influence of bile salts, that are enhancers for nasal absorption of peptide drugs, on the nasal mucosa was also studied from the viewpoint of membrane potential. The nasal mucosa membrane was negatively charged. The membrane charge density (θ^*) was −3.4 mmol/l in KCl solution and −3.5 mol/l in NaCl solution. These membrane potentials of the nasal membrane for isotonic KCl solution were not changed by pretreatment with 1% (w/v) bile salts (sodium glycocholate and dehydrocholate). The membrane potential of nasal mucosa for NaCl and KCl deviated from the simple Donnan equilibrium theorem in the region of low bulk concentration, whereas the membrane potential of the cellulose membrane obeyed the theorem over all the bulk solution concentrations. This difference may be a result of the variation in the amount of ions adsorbed on the nasal mucosa membrane.

Introduction

Peptide drugs 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 (Longenecker, 1987). Many studies of intranasal absorption are however, only concerned with drug permeation to nasal mucosa in vivo. A few studies have reported on physicochemical properties, such as the electric conductance of the nasal mucosa in experimental animals and also on pretreatment with bile salts (Wheatley et al., 1988), but the electric charge of the nasal mucosa in solutions of NaCl and KCl is not yet known. Recently, the electrical charge of the cornea in rabbits has been reported (Rojanasakul and Robinson, 1989).

It is very important from a pharmaceutical point of view to investigate the electric charge of the nasal mucosa in order to understand the permeability of the nasal mucosa for ionic drugs, particularly peptides and proteins. During nasal absorption of peptides, bile salts are used as an absorption promoter (Hirai et al., 1981; Gordon et al., 1985; Maitani et al., 1988). Therefore, it is also important to investigate the membrane potential of nasal mucosa after pretreatment by bile salts.

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The objective of this study is to investigate the effect of bile salts on the nasal mucosa in rabbit by measuring the membrane potential.

Materials and Methods

Materials

All chemicals used were of analytical grade. Sources for some of the chemicals used were as follows: potassium chloride was from Wako Pure Chem. Ind. Ltd; sodium chloride was from Matsunaga Chem. Ind. Ltd; sodium glycocholate (GC) was from Sigma (St. Louis, MO, U.S.A.); and sodium dehydrocholate (DHC) was from Nakarai Chem. Ltd. NaCl and KCl were used after drying under vacuum at 120 °C for 6 h. All solutions were made using water doubly distilled in Pyrex glass. Cellulose membrane was obtained by cutting pieces from seamless cellulose tubing (Viskase Sales Co.). The cellulose membrane was treated by boiling in distilled water for 12 h while the distilled water was replaced several times.

Mucosal preparation

The nasal mucosa used in these experiments was obtained from male New Zealand White rabbits (Saitama Experimental Animal Supply Co.; 3.0–3.5 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 just anterior orbits 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 area 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.

Measurement of the membrane potential

The Ag-AgCl electrode (Tamamushi, 1957) was prepared by winding a platinum wire 5 cm long in a spiral, plating the resulting spiral wire with silver in a solution of $AgK(CN_2)$ (1 g/100 ml) for 6 h. After sufficient washing with distilled water, the plated spiral wire of platinum was further electrolyzed at a current density of 1 mA/cm^2 in 0.1 N HCl for 0.5 h to convert the surface of the Ag into AgCl.

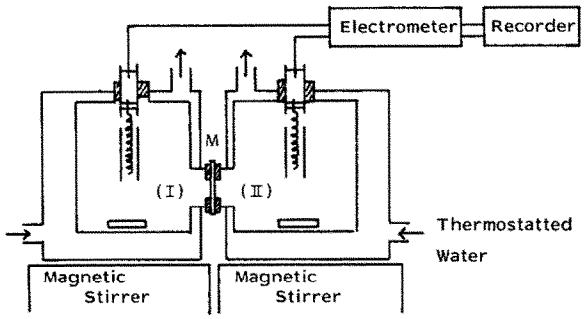

Fig. 1. Apparatus for the measurement of membrane potential. (I) C^{I}, (II) C^{II}; M: nasal mucosa membrane.

The potential difference was measured with a PM-18R electrometer (TOA Electronics Ltd). The membrane potential was measured at 36 °C by inserting Ag-AgCl electrodes directly into the NaCl or KCl solutions of the mucosal and serosal surfaces of nasal mucosa membrane (Fig. 1). Since the potential difference obtained here is the sum of the membrane potential and the electrode potential, the membrane potential was determined by subtracting the electrode potential, $-(RT/F)\ln(a^{I}/a^{II})$, from the potential difference (Hornibrook et al., 1942; Gordon, 1943). The values a^{I} and a^{II} are the activities of chloride ion in solutions I and II, respectively.

The ratio (r) of the mucosal bulk concentration to serosal bulk concentration (NaCl, KCl) was always 2 : 1 ($r = 2$). If not specially mentioned, the bulk solution, C^{I} (rC), was in contact with the mucosal surface which is the ciliated epithelial surface lining the nasal cavity and the bulk solution, C^{II} (C), was in contact with the serosal surface which is the side attached to the nasal cartilage. The total volume of bulk solution in each chamber was 50 ml. The concentrations of the bulk solutions were changed over the range of $10^{-3.4}$–$10^{-1.0}$ mol/l. The membrane potential was measured by changing solutions I and II from a

high concentration to a low one. The solutions were efficiently stirred using a magnetic stirrer and kept at a temperature of 36 °C.

Treatment with bile salts

After the membrane was clamped between the two chambers, the mucosal surface of the nasal mucosa membrane was bathed in a 1% (w/v) solution of bile salts (GC and DHC) for 5 min. After the surface of the nasal mucosa membrane was rinsed with distilled water, the membrane potential was measured.

Theory

The concentrations of the bulk solutions on both sides of the membrane are set at C^{I} (= rC) and C^{II} (= C). Considering that a Donnan equilibrium exists between the membrane surface and the solution in contact with the surface, the concentrations of cations C_{+}^{I*}, C_{+}^{II*} and anions C_{-}^{I*}, C_{-}^{II*} on both membrane surfaces can be calculated.

The membrane potential arising here, which is the algebraic sum of the Donnan potential and diffusion potential, can be represented in the case of a 1-1 valence electrolyte by Eqn 1 (Kobatake et al., 1966; Takeguchi and Nakagaki, 1969).

$$\begin{aligned}\Delta E &= E^{I} - E^{II} \\ &= (RT/F)\Big[-\ln r + \ln\big(C_{+}^{I*}/C_{+}^{II*}\big) \\ &\quad + (2t_{-}^{*} - 1)\ln\Big\{\big(C_{+}^{I*} + t_{-}^{*}\theta^{*}\big) \\ &\quad /\big(C_{+}^{II*} + t_{-}^{*}\theta^{*}\big)\Big\}\Big] \qquad (1)\end{aligned}$$

Here, E^{I} and E^{II} are the potentials of solutions I and II, respectively, θ^{*} is the membrane charge density (mol/l), t_{-}^{*} is the transport number of the anion in the membrane, and r is the concentration ratio of solutions I and II which has a value of 2 in the present experiment. C_{+}^{I*} is calculated as follows in the case of a 1-1 valence electrolyte based on the assumption of a Donnan equilibrium (Takeguchi and Nakagaki, 1969).

$$C_{+}^{I*} = \Big\{-\theta^{*} + \sqrt{\theta^{*2} + 4(rC)^{2}}\Big\}/2 \qquad (2)$$

C_{-}^{I*} can easily be calculated from the condition of electroneutrality as shown in Eqns 3 and 2:

$$C_{-}^{I*} = C_{+}^{I*} + \theta^{*} \qquad (3)$$

C_{+}^{II*} and C_{-}^{II*} can be calculated by setting $r = 1$ in Eqns 2 and 3 for C_{+}^{I*} and C_{-}^{I*}. In a sufficiently high concentration of the solution $C \gg |\theta^{*}|$, Eqn 1 leads to the following equation:

$$\begin{aligned}F\Delta E/RT &= (2t_{-}^{*} - 1)\ln r + 2\{(r-1)/r\} \\ &\quad \times t_{-}^{*}(1 - t_{-}^{*})(\theta^{*}/C) \qquad (4)\end{aligned}$$

Accordingly, t_{-}^{*} can be calculated by plotting the measured values of the membrane potential, ΔE, at high concentration vs $(1/C)$ and extrapolating the plot to the zero value of $(1/C)$.

Results and Discussion

The membrane potential of the nasal mucosa was measured in NaCl or KCl solution by two methods; (1) from the low bulk concentration to the high one, (2) from the high bulk concentration

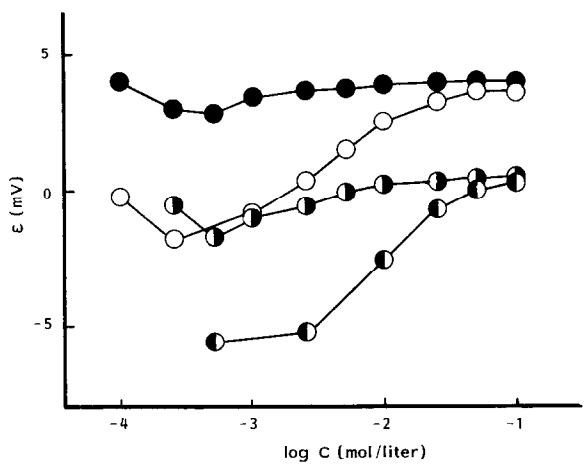

Fig. 2. Plots of membrane potentials of the nasal mucosa for NaCl and KCl against log C when $r = 2$ at 36 °C. (●) Measurement for NaCl from the low bulk concentration to the high one; (○) measurement for NaCl from the high bulk concentration to the low one; (◑) measurement for KCl from the low bulk concentration to the high one; (◐) measurement for KCl from the high bulk concentration to the low one.

to the low one (general method), as shown in Fig. 2. For the low bulk concentration, the membrane potentials did not decrease using method (1), compared with the membrane potentials when using method (2). It is observed that it takes time to reach an equilibrium between nasal mucosa and the solution of low bulk concentration. The membrane potential did not decrease when measuring from the low bulk concentration first, which involves method (1). However, the values of the membrane potentials of cellulose membrane were found to be the same by using two methods. Based on this result, we started to measure the membrane potential from the high bulk concentration to the low one.

The nasal mucosa is not a symmetric membrane but an asymmetric membrane. Asymmetric properties of the nasal mucosa were investigated from the measurement of the membrane potential for NaCl in the normal and reverse position of nasal mucosa between the two glass chambers. Here, the normal position of nasal mucosa means that the bulk concentration in the mucosal surface of the nasal mucosa was always higher than that in the serosal one. The reverse position of nasal mucosa means that the mucosal membrane was attached between two glass chambers in the opposite way. In this case, the bulk concentration in the mucosal surface of the nasal mucosa was lower than that in the serosal one. Comparing the normal and reverse positions of the nasal mucosa, the difference between their membrane potentials was small in the region of high bulk concentration and was not detected at all at the isotonic one as shown in Fig. 3.

Fig. 4 shows the influence of incubation in KCl solution on the membrane potential. Plots show the membrane potentials of the nasal mucosa in KCl solution at 36 °C immediately after dissection and after incubation in KCl ($1 \times 10^{-3.3}$ mol/l) solution for 16 h. The membrane potential of the nasal mucosa that was used immediately after dissecting showed a negative potential in the region of low bulk concentration. However, after incubation in KCl solution at 36 °C for 16 h, the membrane potential of the nasal mucosa was almost 0 mV at all bulk concentrations. This phenomenon may result from the charged component

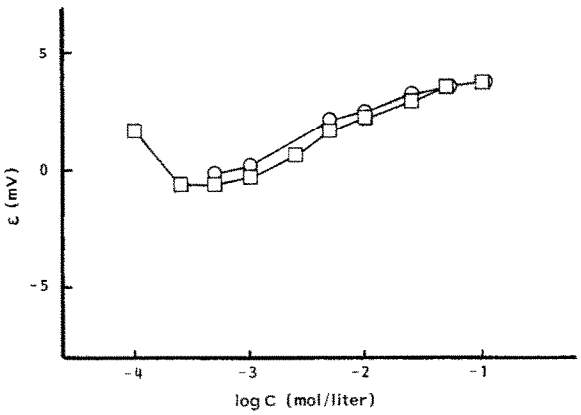

Fig. 3. Change of membrane potentials for NaCl by reversing the mucosal side in the nasal membrane at 36 °C. (□) Normal position of mucosal side facing C^{II}; (○) reverse position of mucosal side facing C^{I}.

of the membrane being dissolved into the solution or the K^+ being sufficiently adsorbed on the nasal mucosa to neutralize the membrane charge. This result appeared to prove that the electrical property of nasal mucosa was not changed during one series of measurements from $10^{-1.0}$ to $10^{-3.4}$ mol/l of bulk solution.

From the membrane potentials, ΔE, of cellulose membrane, the transport number of chloride ion, t^*_-, in the membrane was first determined by plotting the resulting potential, ΔE, vs the reciprocal of the concentration, $1/C$, from the

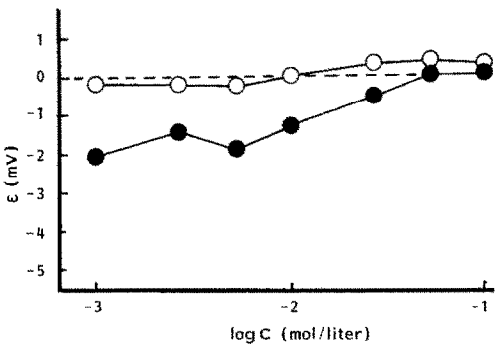

Fig. 4. Change of membrane potentials of nasal mucosa after incubation in KCl solution at 36 °C. (●) Measured immediately after dissecting the nasal mucosa; (○) measured after incubation of nasal mucosa in $10^{-3.3}$ mol/l of KCl solution for 16 h at 36 °C.

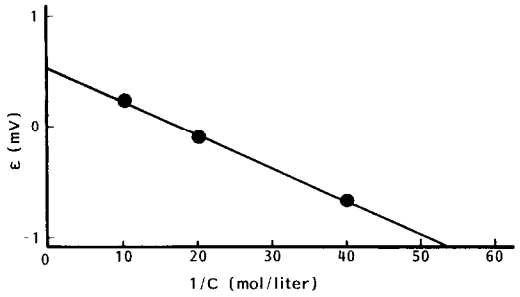

Fig. 5. Plots of membrane potential of cellotube for KCl against $1/C$ at 36 °C.

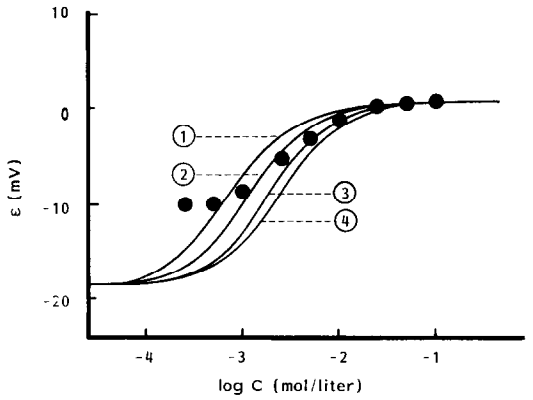

Fig. 6. Theoretical curves of membrane potentials of cellotube in KCl for various values of θ^* at 36 °C. (1) $\theta^* = -1.0\times10^{-3}$, (2) $\theta^* = -2.0\times10^{-3}$, (3) $\theta^* = -3.0\times10^{-3}$, (4) $\theta^* = -4.0\times10^{-3}$. Experimental values are also plotted. From the best fit, the membrane charge density, θ^*, was found to be -2.8×10^{-3}.

limiting potential obtained when $1/C$ approaches 0 according to Eqn 4, as shown in Fig. 5.

The transport number of Cl^- in KCl and NaCl of the membrane of cellulose tube, 0.52 and 0.62, are almost equal to those in water, 0.52 and 0.59, respectively. It is considered that the ratio of the mobility of anion to that of cation was hardly affected in the membrane of cellulose tube.

By using the obtained transport number of Cl^- in Table 1, theoretical curves can be drawn for various θ^* values from -1×10^{-3} to -4×10^{-3} mol/l (Fig. 6). The best fitting theoretical curves were drawn for KCl and NaCl as shown in Fig. 7, together with the experimental values for the cellulose membrane. The θ^* values for these best fits were $\theta^* = -2.8$ and -3.1 mmol/l in KCl and NaCl, respectively. The agreement is good except in the region of very low electrolyte concentration.

Fig. 8 shows the best fitting theoretical curves calculated according to Eqn 1 and the experimental values for the nasal mucosa in KCl and NaCl. From the fitting of the experimental data to the theoretical curves, the values of $\theta^* = -3.4$ mmol/l in KCl and -3.5 mmol/l in NaCl were obtained in the nasal mucosa. Therefore, the nasal mucosa was negatively charged and allows preferential passage of positive ions with respect to negative ions. The charge of the nasal mucosa may result from negatively charged lipid and the sial acid of glycoprotein in the mucus of the nasal mucosa. The membrane potential of the nasal mucosa in the region of low bulk concentration deviated from the theoretical curves.

The results obtained are listed in Table 1. The values of t_+^* in NaCl for the membrane of cellulose tube and the nasal mucosa were lower than those in KCl for these membranes. This may result from the difference in ionic radius of the hydrated ions. The ionic radius of hydrated Na^+ is greater than that of K^+ (Monk, 1961).

Fig. 9 shows the membrane potential before and after pretreatment with 1% (w/v) solution of GC or DHC for 5 min. The concentration of bile

TABLE 1

Values of $t_\pm^*$ and θ^* obtained from the measurements of membrane potential

		t_-	$t_\pm^*$	$t_\pm^*/t_-$	t_+^*	θ^* (mM/l)
Cellotube	KCl	0.52	0.52	1.00	0.48	−2.8
	NaCl	0.59	0.62	1.04	0.38	−3.1
Nasal mucosa	KCl	0.52	0.50	0.96	0.50	−3.4
	NaCl	0.59	0.60	1.02	0.40	−3.5

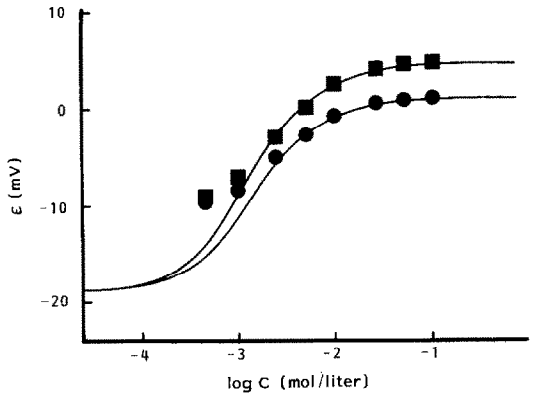

Fig. 7. Plots of membrane potentials of cellulose membrane against log C when $r = 2$ at 36 °C. (■) NaCl, (●) KCl.

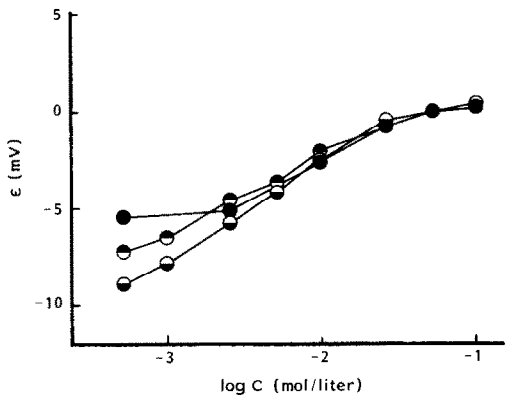

Fig. 9. Effects of bile salts on membrane potentials of the nasal mucosa for KCl at 36 °C. (●) No treatment; (◒) pretreatment with 1% (w/v) solution of GC for 5 min; (◓) pretreatment with 1% (w/v) solution of DHC for 5 min.

salts were chosen to be 1% (w/v) since this concentration was used as an absorption promoter in intranasal absorption in vitro (Murakami et al., 1984; Wheatley et al., 1988). There is a slight difference of the membrane potential values before and after pretreatment by bile salt solution at the low concentration of KCl. GC made the membrane potential even more negative than DHC. Wheatley et al. (1988) reported that GC increased the conductance of ovine nasal mucosa with pretreatment but DHC showed no effect on conductance. This conductance result is inconsistent with our results from the membrane potential that GC affects the nasal mucosa more than DHC. The result suggested that the anionic bile salt was absorbed in the nasal mucosa and that the membrane potential then became more negative at the low concentration of KCl. The other interpretation is that epithelial cells, which represent a barrier to permeation, were drawn out by pretreatment with the bile salt (Hersey and Jackson, 1987). Bile salts make permeability for drugs in the nasal mucosa increase dramatically in vivo and vitro. However, it appeared that a 1% (w/v) solution of GC and DHC did not effectively change the membrane potential at the isotonic concentration.

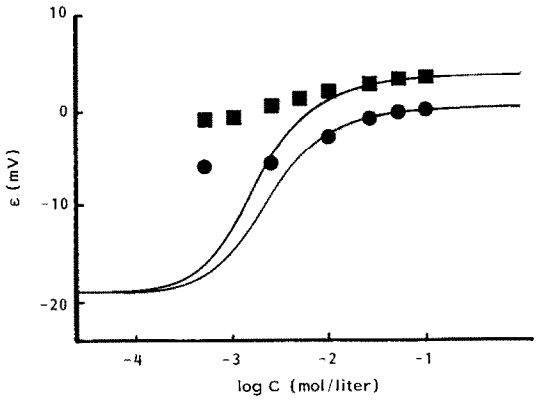

Fig. 8. Plots of membrane potentials of nasal mucosa against log C when $r = 2$ at 36 °C. (■) NaCl, (●) KCl.

Conclusions

The study based on the membrane potential was conducted in the nasal mucosa. The study showed the nasal mucosa membrane carries a net negative charge. The nasal mucosa is not a symmetric membrane but an asymmetric membrane. However, asymmetric properties of the nasal mucosa could not be detected from the measurement of the membrane potential. The incubation of the nasal mucosa in KCl solution made the membrane potential almost 0 mV, indicating that the effective charge of the membrane was negated by incubation. The membrane potential of nasal mucosa became slightly more negative by pretreatment with bile salt solution. The result suggested that the bile salt had penetrated into the nasal mucosa and that the negative membrane charge

density increased. The knowledge obtained from this study may provide strategies for transepithelial delivery of drugs especially those with peptides which are amphoteric.

Acknowledgement

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