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Mechanisms for the enhancement of the nasal absorption of insulin by surfactants

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Summary

The mechanisms of the promoting effect of surfactants on the nasal absorption of insulin was investigated in rats. The promoting effect of several non-ionic surfactants, sodium lauryl sulfate and saponin, were paralleled by their abilities to lyze the rabbit erythrocyte and to release protein from the nasal mucosa, whereas the promoting effect of bile acid salts was attributed not only to their direct effect on the nasal mucosa, but also to their inhibitory effect on proteolytic enzymes. Mucosal alterations, observed by scanning electron microscopy, were reversible and the membrane was relatively rapidly restored. Bile acid salts were found to be less irritative to the nasal mucosa than non-ionic ether type surfactants.

Introduction

The nasal route for drug administration has been of great interest and absorption from this route has been studied widely in order to improve drug bioavailability. Studies have shown that the nasal administration of propranolol, which is absorbed inefficiently and variably after oral dosage, was superior to oral administration from the standpoint of bioavailability and was as effective as intravenous administration in rats, dogs and humans (Hussain et al., 1979; 1980a and b). In addition, nasal administration of insulin to dogs, humans and rats resulted in significant hypoglycemia (Hirai et al., 1978; 1981a and b; Yokosuka et al., 1977). However, to enhance the insulin bioavailability, it was necessary to add a surfactant (e.g. non-ionic ether type surfactants, bile acid salts, saponin) to the preparation.

The purpose of the present study was to clarify the mechanisms of the promoting effect of the surfactants on the nasal absorption of insulin and to investigate the morphological change of the nasal mucosa by a scanning electron microscope following the nasal administration of insulin solution with a surfactant.

Materials and methods

Materials

Crystalline pork insulin [1] and the surfactants, listed in Tables 1 and 2, were used as supplied. All other solvents and reagents were of analytical grade.

Nasal absorption

Male Sprague–Dawley rats weighing 200–300 g were used. An operation for the in vivo nasal absorption study was described previously (Hirai et al., 1981a). Twenty minutes after the operation, 0.1 ml/kg of insulin preparation (10 U/kg) was administered to the nasal cavity by means of a micropipette through the nostril, which was closed immediately after the administration with an adhesive agent [2]. After the administration, 0.2 ml of blood was taken periodically from the tail vein. The plasma was separated by centrifugation at 3000 rpm and the plasma glucose level was estimated by the *o*-toluidine method (Hyvärinen and Nikkilä, 1962). The decrement of the plasma glucose level from 0 to 4 h (D, total decrease) was calculated according to the equation described previously (Hirai et al., 1981b).

Erythrocyte hemolysis

About 10 ml of whole blood was withdrawn from the heart of a rabbit and was immediately stirred well by a glass rod to remove the fibrins. Then, 5 ml of the non-fibrinated blood was centrifuged at 2000 rpm for 5 min. After removing the supernatant solution, the erythrocytes were suspended in 3 ml of physiological saline and the suspension was centrifuged. The same procedure was repeated 3 times. The washed erythrocytes were diluted with physiological saline to produce 50 ml of the erythrocyte suspension. Five ml of the test solution of a surfactant, prepared by dissolving it in physiological saline at various concentrations, was placed in a 10 ml test tube, to which was added 0.2 ml of the erythrocyte suspension. After 5 min incubation at 25°C, the mixture was cooled in ice-water and was centrifuged for 5 min at 2000 rpm. The absorbance of the supernatant was measured at 540 nm to determine the percentage of hemolysis.

Protein release from the nasal mucosa

Release of protein from the nasal mucosa caused by a surfactant was determined by a modification of the in situ recirculation technique (Hirai et al., 1981a). The 0.1% surfactant saline solution was perfused from the posterior side of the nasal cavity through the nostrils at a rate of 2 ml/min. The perfusate was collected for 10 min and the amount of protein was determined by the Hartree method (1972).

Stability of insulin and determination of leucine aminopeptidase activity in the nasal mucosa homogenate

After the rats were anesthetized with ether and decapitated, the nasal mucosa on

[1] Shimizu Seiyaku Co., Ltd., Shizuoka, Japan.

[2] Aron Alpha A, Sankyo Co., Ltd., Tokyo, Japan.

the septal cartilage was isolated by incising the frontal bone and was homogenized in a 10-fold volume of cold saline in a glass–teflon homogenizer.

The homogenate was centrifuged at $9000 \times g$ for 10 min at 5°C and the supernatant was stored in a freezer until analysis. One ml of the mixture containing 0.2 ml of the supernatant of the nasal mucosa homogenate, 40 mU of insulin, 0.04 M of pH 7.4 phosphate buffer and 1% of a surfactant, such as sodium glycocholate or polyoxyethylene 9 lauryl ether, was incubated at 37°C for 60 min. At specific times, 0.1 ml of the mixture was withdrawn and added to 1 ml of 0.1 N HCl to terminate the enzymatic reaction. The residual amount of insulin in the mixture was determined by radioimmunoassay [3].

Leucine aminopeptidase activity of the nasal mucosa homogenate was estimated according to the modified Goldbarg method (Takenaka and Takahashi, 1962). Thirty μl of the supernatant of the tissue homogenate was added to 0.5 ml of a substrate solution, prepared by dissolving 20 mg L-leucylnaphtylamide hydrochloride in 100 ml of 0.05 M phosphate buffer (pH 7.0). The mixture was incubated at 37°C for 15 min. After the addition of 1 ml of 0.2 N HCl, to terminate the enzymatic hydrolysis, 1 ml of 4% *p*-dimethylaminobenzaldehyde ethanol solution was added to the mixture. After 20 min, the absorbance of the mixture was measured at 450 nm. The inhibitory effect of a surfactant on the leucine aminopeptidase activity of the nasal mucosa was determined at concentrations from 0 to 1% surfactant.

Recovery of the hyperabsorptive state produced by surfactant

The 0.1 mg/kg of 1% surfactant saline solution, such as sodium glycocholate or polyoxyethylene 9 lauryl ether, was administered to the nasal cavity of unanesthetized rats through the nostril by means of a micropipette. At various time periods after administration of these promoters, the insulin solution without promoter at a dose of 10 U/kg was administered by a method described previously (Hirai et al., 1981a). Thereafter, the blood was taken periodically from the tail vein and the plasma glucose level was estimated.

Scanning electron microscopic observations

Rats were killed at 2, 4 and 24 h after the nasal administration of 1% sodium glycocholate or polyoxyethylene-9-lauryl ether saline solution, at a volume of 0.1 ml/kg. The nasal mucosa was removed, and fixed on a rubber plate, and its surface washed with cold saline solution. Then, the tissue sample was immersed for 5 h in a 2.5% glutaldehyde solution for fixation and stored overnight in a 6% sucrose solution. The sample was dehydrated by soaking successively in 50, 70, 90, 100% ethanol and 100% acetone for 20 min. Then, a piece was cut from the air-dried sample, fixed on a brass-block with a conductive silver paint [4], coated with gold by high vacuum evaporator [5], and examined by a scanning electron microscope [6].

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3 Radiochemical Center, Amersham, England.
4 Dotite D-550, Fujikura Kasei, Co., Ltd., Tokyo, Japan.
5 JEE-48, Japan Electron Optics Lab., Co., Ltd., Tokyo, Japan.
6 JSM-2, Japan Electron Optics Lab., Co., Ltd., Tokyo, Japan.

In order to investigate the effect of a surfactant on the nasal mucosa in subchronic use, the insulin solution (2 U/kg) with 1% sodium glycocholate or polyoxyethylene 9 lauryl ether was administered to the rat nostril 3 times daily for one month at a volume of 0.1 ml/kg. Sixteen hours after the last administration, rats were killed and the nasal mucosa was removed and examined by a scanning electron microscope.

Results

In order to investigate the details of the promoting effect of surfactants on the nasal absorption of insulin, the influence of the average number of ethylene oxide units in polyoxyethylene lauryl ether on the plasma glucose concentration was examined. Typical examples of the change in plasma glucose level after nasal administration of the insulin solution (pH 7.4) containing 0.3% surfactant are shown in Fig. 1. The insulin solution without surfactant produced slight hypoglycemia throughout the experimental period, whereas the addition of a surfactant significantly decreased the plasma glucose level. Fig. 2 shows the relation between the number of ethylene oxide units in polyoxyethylene lauryl ether and the promoting effect on nasal absorption. The decrement of plasma glucose level showed a maximum of 55.5% with polyoxyethylene 9 lauryl ether and diminished gradually with smaller or larger numbers of ethylene oxide unit. The addition of 0.3% polyoxyethylene 3 or 50 lauryl ether showed a less than 10% decrement.

To examine the effects of surfactants on biomembranes, their hemolytic activity and protein-releasing effect were investigated. Fig. 3A shows the hemolytic activity, which is the reciprocal of minimum concentration to cause 100% hemolysis versus the number of ethylene oxide units in polyoxyethylene lauryl ether and Fig. 3B shows the amount of protein released from the nasal mucosa versus the number of ethylene oxide units. Both hemolytic and protein-releasing effects of these surfac-

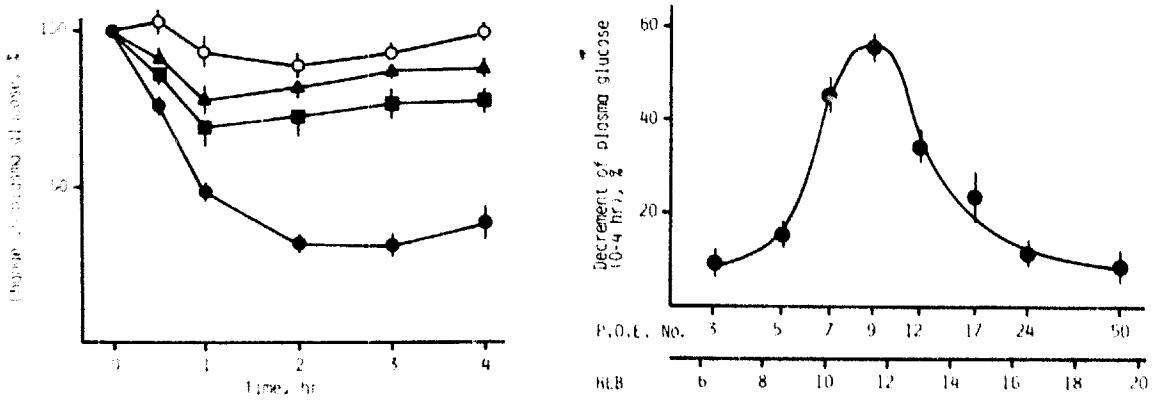

Fig. 1. change in plasma glucose after nasal administration of insulin (10 U/kg) in rats (pH 7.4, 0.3% surfactant). The data are expressed as mean ± S.E. ○, no surfactant (n = 4); ▲, polyoxyethylene 5 lauryl ether (n = 5); ●, polyoxyethylene 9 lauryl ether (n = 7); ■, polyoxyethylene 17 lauryl ether (n = 4).

Fig. 2. Promoting effect of polyoxyethylene lauryl ethers on the nasal absorption of insulin (10 U/kg) in rats (pH 7.4, 0.3% surfactant). The data are expressed as mean ± S.E. (n = 4–7).

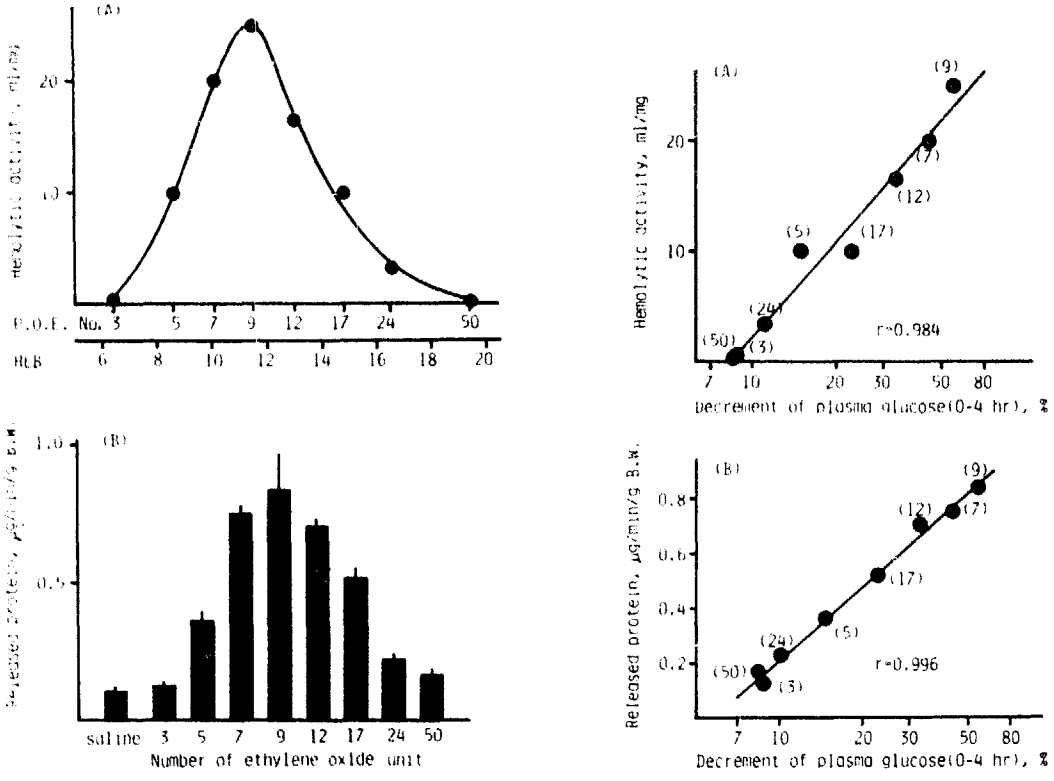

Fig. 3. A: effect of polyoxyethylene lauryl ethers on erythrocyte hemolysis. The data are expressed as mean of 3 experiments. B: effect of polyoxyethylene lauryl ethers (0.1%) on protein released from the nasal mucosa of rat. The data are expressed as mean ± S.E. (n = 4).

Fig. 4. A: relation between hemolytic activity and promoting effect of polyoxyethylene lauryl ethers. B: relation between protein releasing effect and promoting effect of polyoxyethylene lauryl ethers. Numbers in parentheses refer to the number of ethylene oxide units in polyoxyethylene lauryl ether.

tants on the biomembrane were at a maximum with polyoxyethylene 9 lauryl ether and decreased as the number of ethylene oxide units increased or decreased from 9. Fig. 4A and B show the hemolytic activity and the protein releasing effect versus the decrement of plasma glucose level within 0 to 4 hours: the degree of correlation is high, with a correlation coefficient of 0.984 and 0.996, respectively. These results suggest that the nasal absorption of insulin can be promoted by a surfactant having a strong effect on biomembranes.

Tables 1 shows the hemolytic activity and the protein releasing effect of various kinds of surfactants, as well as the decrement of plasma glucose level by the addition of 1% surfactant which has been reported previously (Hirai et al., 1981b). Non-ionic ether type surfactants, such as polyoxyethylene lauryl ether, cetyl ether, stearyl ether and nonylphenyl ether, were found to have marked effects on the nasal mucosa. Non-ionic ester type surfactants were found to show lesser activities. There existed a good correlation between the effect on the biomembrane and the absorption-promoting effect of the non-ionic surfactants. A good correlation was also found for the anionic surfactant, sodium lauryl sulfate, and saponin. Bile acid salts, such as

Table 1

Effects of surfactants on protein release from the nasal mucosa and erythrocyte hemolysis

Surfactant	Released protein [a] (μg/min/g (body weight)	Hemolysis [b] (ml/mg)	D [c] (%)
Saline	0.11 ± 0.02		
P.O.E. 5 [i] lauryl ether [d]	0.36 ± 0.03	10.0	59.3 ± 3.0
P.O.E. 9 lauryl ether [d]	0.83 ± 0.13	25.0	60.9 ± 2.9
P.O.E. 10 cetyl ether [d]	0.68 ± 0.02	25.0	64.0 ± 1.3
P.O.E. 10 stearyl ether [d]	0.58 ± 0.01	8.3	53.3 ± 2.9
P.O.E. 10 nonylphenyl ether [d]	0.75 ± 0.04	25.0	57.3 ± 0.5
P.O.E. 10 monolaurate [d]	0.26 ± 0.03	3.3	35.1 ± 6.9
P.O.E. 10 monostearate [d]	0.09 ± 0.01	0.2	16.6 ± 3.2
P.O.E. 40 monostearate [d]	0.17 ± 0.01	0.1	5.5 ± 5.3
P.O.E. 20 sorbitan monooleate [e]	0.17 ± 0.01	0.07	6.4 ± 3.2
P.O.E. 50 hydrogenated castor oil [f]	0.21 ± 0.02	0.1	10.3 ± 4.6
Sodium laurylsulfate [g]	1.99 ± 0.07	14.3	52.9 ± 1.4
Sodium taurocholate [h]	0.29 ± 0.00	2.5	56.7 ± 3.3
Sodium cholate [h]	0.36 ± 0.03	3.3	53.1 ± 4.8
Sodium glycocholate [h]	0.21 ± 0.02	3.3	53.1 ± 2.7
Saponin [g]	0.77 ± 0.01	20.0	53.9 ± 2.5

[a] The concentration of surfactant in the perfused solution was 0.1%. Results are expressed as mean ± S.E. of 4 experiments. [b] Each value, the reciprocal of minumum concentration of 100% hemolysis, is expressed as mean of 3 experiments. [c] The decrement of plasma glucose level (D) was obtained from previous paper (Hirai et al., 1981b). [d] Nihon Emulsion Co., Ltd., Tokyo, Japan. [e] Kao-Atlas Co., Ltd., Tokyo, Japan. [f] Nikko Chemical, Ltd., Tokyo, Japan. [g] Wako Pure Chemical Ind., Ltd., Osaka, Japan. [h] Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. [i] The number of ethylene oxide units (P.O.E.) are denoted as P.O.E. n.

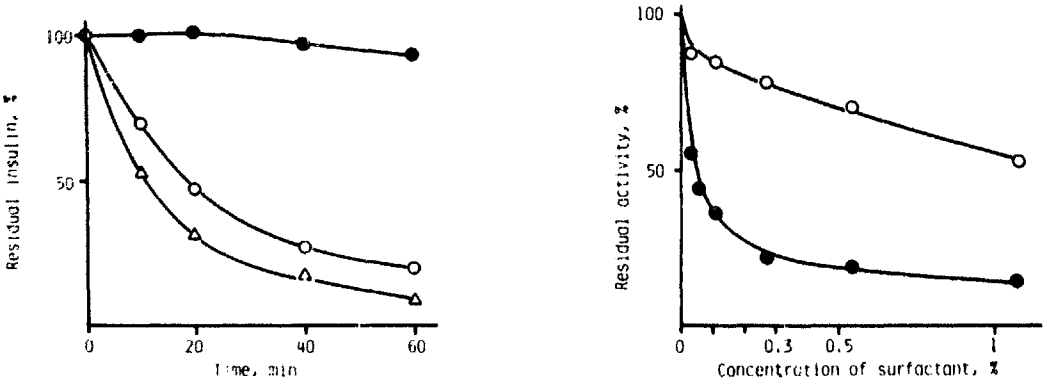

Fig. 5. Effect of surfactants on the degradation of insulin in the rat nasal mucosa. The data are expressed as mean of 3 experiments. △, no surfactant; ●, 1% sodium glycocholate; ○, 1% polyoxyethylene 9 lauryl ether.

Fig. 6. Effect of surfactants on the activity of leucine aminopeptidase in the rat nasal mucosa. The data are expressed as mean of 3 experiments. ●, sodium glycocholate; ○, polyoxyethylene 9 lauryl ether.

Table 2

Inhibitory effect of surfactants on the leucine aminopeptidase activity in the nasal mucosa

Surfactant	Inhibition (%)
P.O.E. 5 [d] butyl ether [a]	14.8 (8.5–18.3)
P.O.E. 10 butyl ether [a]	13.3 (10.3–15.8)
P.O.E. 20 butyl ether [a]	−1.7 (−3.2–3.0)
P.O.E. 3 lauryl ether [a]	8.2 (6.9–9.0)
P.O.E. 5 lauryl ether	19.7 (16.8–25.3)
P.O.E. 9 lauryl ether	22.7 (19.6–28.2)
P.O.E. 10 lauryl ether [a]	13.6 (11.0–18.0)
P.O.E. 20 lauryl ether [a]	19.5 (16.9–22.5)
P.O.E. 10 cetyl ether	12.2 (9.7–14.8)
P.O.E. 20 stearyl ether [a]	−2.3 (−5.6–1.1)
P.O.E. 24 cholesteryl ether [b]	16.3 (12.2–20.8)
P.O.E. 40 monostearate	−16.9 (−19.5–12.5)
P.O.E. 10 monolaurate	20.1 (15.7–28.3)
P.O.E. 20 sorbitan monooleate	0.8 (−2.5–1.2)
P.O.E. 50 hydrogenated castor oil	3.3 (1.0–4.6)
Sodium laurylsulfate	98.3 (97.4–100.0)
Sodium oleate [c]	96.8 (94.9–99.7)
Sodium taurocholate	87.4 (80.7–96.3)
Sodium cholate	84.9 (79.2–89.2)
Sodium glycocholate	87.2 (80.5–93.2)
Saponin	36.9 (28.6–42.7)

The concentration of surfactant in the incubation medium was 0.27%. Results are expressed as mean of 3 experiments and range (in parentheses).

[a] Nihon Emulsion Co., Ltd., Tokyo, Japan.

[b] American Cholesterol Products Inc., New Jersey, U.S.A.

[c] Wako Pure Chemical Ind., Ltd., Osaka, Japan.

[d] The number of ethylene oxide units are denoted as P.O.E. n.

taurocholate, cholate and glycocholate, showed lesser effects on both hemolytic activity and protein release, though the absorption promoting effects were significant and almost the same as those of non-ionic ether type surfactants, sodium lauryl sulfate and saponin.

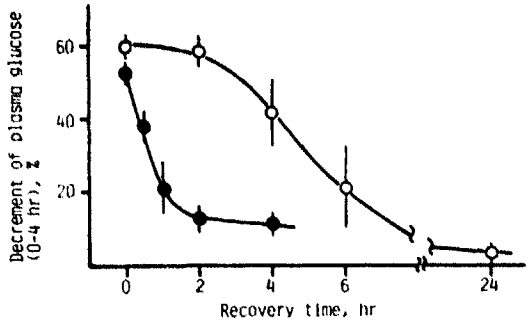

Fig. 7. Effect of recovery time on the hyperabsorptive state of the rat nasal mucosa. The data are expressed as mean ± S.E. (n = 4–9). ●, sodium glycocholate; ○, polyoxyethylene 9 lauryl ether.

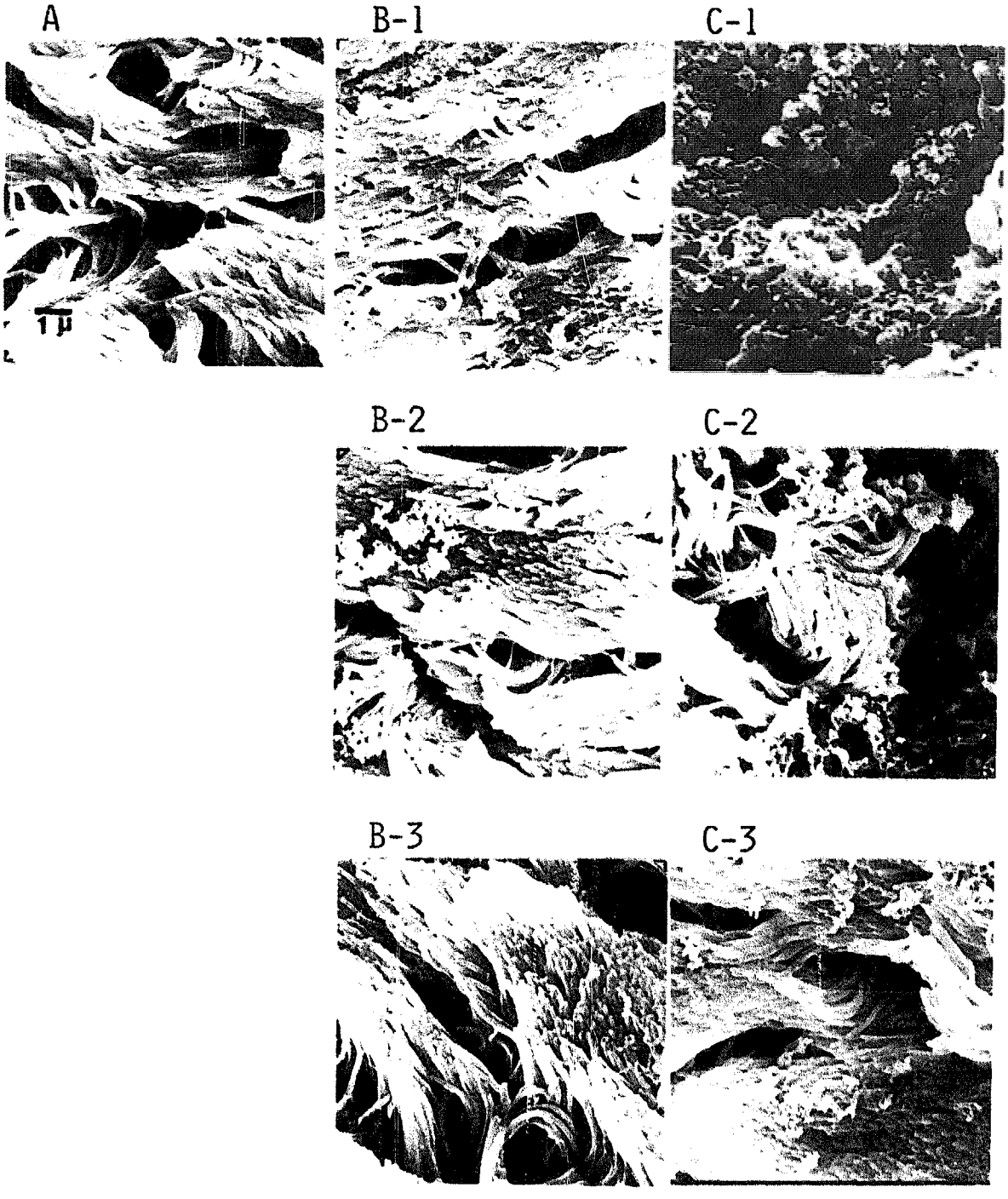

Fig. 8. Scanning electron micrographs of the rat nasal mucosa treated with surfactants. A, control; B-1, B-2 and B-3, 2, 4 and 24 h after nasal administration of 1% sodium glycocholate; C-1, C-2 and C-3, 2, 4 and 24 h after nasal administration of 1% polyoxyethylene 9 lauryl ether, respectively. (×5000).

The effect on the enzymatic hydrolysis of insulin was examined, as another aspect of the effects of bile acid salts. Fig. 5 shows the time course of the residual amount of insulin in the homogenate of the nasal mucosa with and without sodium glycocholate or polyoxyethylene 9 lauryl ether. Insulin in the tissue homogenate was

subjected to rapid degradation and only 9% of the initial insulin remained at 60 min. The addition of polyoxyethylene 9 lauryl ether resulted in a slight inhibition of the enzymatic degradation of insulin, whereas sodium glycocholate significantly inhibited the enzymatic hydrolysis; the residual amount of insulin was 92% at 60 min incubation.

The effects on the activity on the nasal mucosa of leucine aminopeptidase, which is known to cleave the B-chain of insulin, were determined with sodium glycocholate or polyoxyethylene 9 lauryl ether in the tissue homogenate. As shown in Fig. 6, the activity of leucine aminopeptidase decreased with increasing concentrations of the surfactants. A more marked effect was found when sodium glycocholate rather than polyoxyethylene 9 lauryl ether was used. Table 2 presents data on the inhibitory effect of various kinds of surfactants on the activity of leucine aminopeptidase. Although the addition of 0.27% non-ionic surfactants to the tissue homogenate resulted in less than 20% inhibition on the peptidase activity, bile acid salts were found to cause more than 80% inhibition. These results suggest that bile acid salts affect not only the permeability of the nasal mucosa but also the activity of proteolytic enzyme; the nasal absorption of insulin is promoted by both these effects.

To determine the rate of recovery from the induced hyperabsorptive state by the surfactant, 1% surfactant solution of sodium glycocholate or polyoxyethylene 9 lauryl ether was administered to the nasal cavity and, after predetermined time intervals, an insulin solution without a surfactant was administered. Fig. 7 shows the time course of recovery from the hyperabsorptive state. As the time interval between administration of the surfactant and that of the insulin increased, the nasal absorption of insulin decreased to the level without surfactant. The recovery of hyperab-

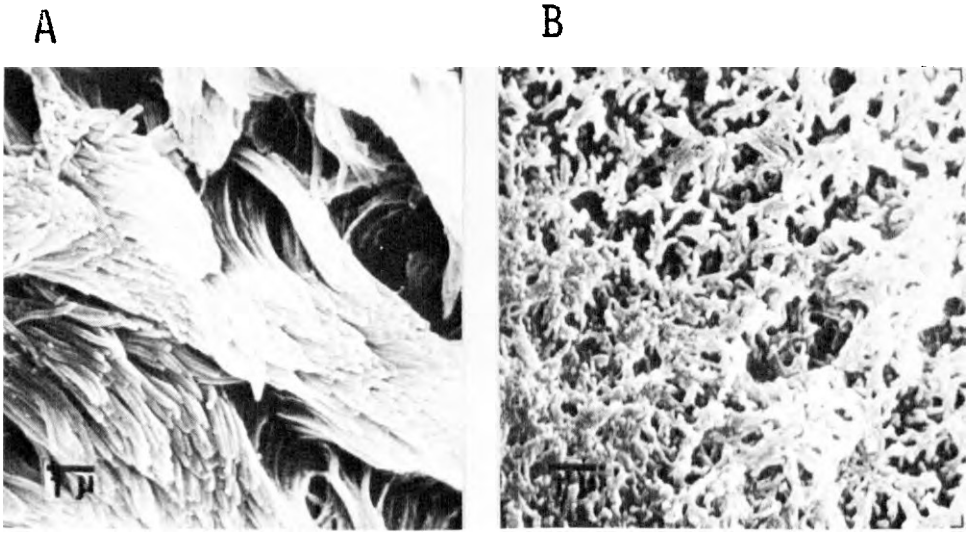

Fig. 9. Scanning electron micrographs of the rat nasal mucosa after successive administration of insulin preparation (2 U/kg×3 times/day, 1 month). A, with 1% sodium glycocholate; B, with 1% polyoxyethylene 9 lauryl ether. (×5000).

sorptive state caused by sodium glycocholate was significantly faster than that caused by polyoxyethylene 9 lauryl ether.

Fig. 8 shows the surface of the nasal mucosa observed by scanning electron microscopy before and 2, 4 and 24 h after the nasal administration of 1% sodium glycocholate or polyoxyethylene 9 lauryl ether. Villi on the nasal mucosa 2 h after the administration of sodium glycocholate were observed to be slightly denuded at the tip but gradual recovery was observed with the passage of time and was complete after 24 h. In contrast, the alteration of the nasal mucosa after administration of polyoxyethylene 9 lauryl ether was significant and complete restoration was not observed at 24 h.

To investigate the subchronic effects, an insulin solution with the surfactant was administered to rats 3 times a day for one month and the nasal mucosa was examined by scanning electron microscopy. As shown in Fig. 9, there was a morphological change in the microvilli of the nasal mucosa after administration of the insulin solution with polyoxyethylene 9 lauryl ether, whereas no change was observed with sodium glycocholate. This result suggests that sodium glycocholate is less irritative than polyoxyethylene 9 lauryl ether on the nasal mucosa.

Discussion

The effects of surfactants on drug absorption have been the object of many studies. A number of these were reviewed by Gibaldi (1970) and Gibaldi and Feldman (1970). They discussed the following as possible principal mechanisms: effects on the solubility and dissolution rate of a drug, effects on the gastric emptying and intestinal transport, drug–surfactant interactions, the consequence of all of the above factors for the passage through the intestinal membrane, and direct effects of surfactants on the permeability of biomembranes. In the present work, we attempted to examine the mechanism of the promoting effect of surfactants on the nasal absorption of insulin, by focussing on the membrane permeation process.

In order to clarify the relation between the absorption-promoting effect of surfactants and their effect on biomembranes, their hemolytic activity and their protein-releasing effect from the nasal mucosa were examined. Gullikson et al. (1977) reported that the relative effects of anionic surfactants on water transport were paralleled by their abilities to lyze erythrocytes, a membrane model. Feldman et al. (1973) demonstrated that sodium taurodeoxycholate accelerated the release of total phosphorus, phospholipid and protein from the everted rat small intestine at concentrations above the critical micelle concentration, and the effects of surfactants on the biomembrane was correlated with an increase in permeability of the intestine to phenol red. In our studies, a good correlation was also observed between the effects on the biomembrane and the absorption-promoting effect of the surfactants, especially the non-ionic surfactants, sodium lauryl sulfate and saponin. This suggests that the effect of the surfactant on the permeability of the nasal mucosa to insulin may be due to the perturbation or disorder of the structural integrity of the nasal mucosa caused by the surfactant.

Bile acid salts, on the other hand, showed milder effects on the biomembrane,

that is, lower hemolytic activity and less protein release than those of other surfactants, in spite of nearly the same absorption-promoting effects. Therefore, an additional mechanism for the absorption-promoting effect of bile acid salts was investigated.

It is well known that a polypeptide such as insulin is subjected to degradation by proteolytic enzymes during passage through the mucosal membrane (Klostermeyer and Humbel, 1966). This reaction has limited the development of non-parenteral application as a practical route of administration. Dierschy (1967) has reported that the addition of bile acid salts to tissue preparations of the small bowel resulted in the inhibition of ATPase activity and almost every process involving oxygen utilization, as well as the synthesis of protein from amino acids, of sterol and fatty acids from acetate, and of triglyceride from glucose and fatty acids. Thus, the effect of surfactants on the activity of proteolytic enzymes in the nasal mucosa was examined. The addition of surfactants, especially bile acid salts, resulted in a significant inhibition of the enzymatic degradation of insulin and of the enzymatic activity of leucine aminopeptidase, which rapidly breaks down the B-chain of insulin from the hydrophobic N-terminal end (Smith et al., 1958). The results suggest that the inhibitory effect of a surfactant on the proteolytic degradation of insulin in the nasal mucosa may be one possible mechanism to explain the absorption-promoting effect of a surfactant.

The results of the time course of the nasal absorption of insulin and the scanning electron microscopy studies indicate that the functional and structural changes of the nasal mucosa caused by the surfactants are reversible and return to normality occurs relatively soon. These phenomena have been already reported from investigations in which the effects of non-ionic surfactants (Davis et al., 1970), anionic surfactants (Khalafallah et al., 1975; Briseid et al., 1974) and bile acid salts (Harries and Sladen, 1972) on the absorption of water-soluble antibiotics, phenolsulfonphthalein, pralidoxime, electrolytes and monosaccharides in the gastrointestinal tract of dogs, humans and rats have been studied.

After a one-month application of insulin preparation with the surfactant to the nasal cavity, no significant abnormalities were found in the case of sodium glycocholate but slight morphological changes of the nasal mucosa were observed in the case of polyoxyethyiene 9 lauryl ether. The latter might be due to both a direct effect of the surfactants on the nasal mucosa and a difference in the reversibility from the mucosal alteration caused by the surfactant.

On the basis of these experiments, it would appear that nasal administration of insulin with promoter may have a useful clinic effect. However, further detailed chronic toxicity testing will be required for the confirmation of safety of this procedure before clinical application, even when bile acid salts are used as a promoter.

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