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# Effect of surfactants on the nasal absorption of insulin in rats

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## Summary

The nasal administration of insulin preparation to rats resulted in dose-dependent hypoglycemia. The absorption of insulin through the nasal mucosa was enhanced when a surfactant, among various non-ionic, anionic and amphoteric surfactants, including the bile acid salts, saponin and peptidelipid (surfactin), was added to the insulin solution. Among the non-ionic surfactants, the addition of an ether type having a HLB(hydrophile–lipophile balance) value from 8 to 14 was found to produce the highest promoting effect on the nasal absorption of insulin. The bioavailability of nasally administered insulin with the surfactant was estimated to be about 30% by comparing the hypoglycemic effect with that obtained after intravenous administration.

## Introduction

It is well known that insulin, a pancreatic peptide hormone, is barely absorbed after administration by non-parenteral routes because of its high molecular weight and its degradation by proteolytic enzymes. So, a high non-parenteral dose of insulin is necessary to produce the same pharmacological effect as that given parenterally (Shichiri et al., 1975; Wigley et al., 1971; Crane et al., 1968).

In our previous reports (Hirai et al., 1978; Yokosuka et al., 1977), it was shown that the nasal administration of insulin to dogs and humans resulted in a significant increase of the blood immunoreactive insulin level with remarkable hypoglycemia. Moreover, the nasal absorption of insulin in dogs and humans was enhanced by the addition of a promoter, such as sodium glycocholate, to the insulin solution.

The purpose of the present study was to investigate in more detail the nasal absorption of insulin in rats and to screen for absorption-promoting agents for the nasal absorption of insulin among various kinds of surfactants.

## Materials and methods

*Materials and preparations*

Crystalline pork insulin [1] was dissolved in 0.01 M isotonic acetate buffer (below pH 6.0) or in 0.01 M isotonic phosphate buffer (above pH 6.0) for intranasal application at concentrations ranging from 2 U/0.1 ml to 40 U/0.1 ml. Surfactants, listed in Tables 1 and 2, were added to the insulin solution at various concentrations.

*Animal experiments*

Male Sprague–Dawley rats weighing 200–300 g were fasted for about 16 h prior to the experiments and were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg. The operation for the nasal absorption study was described previously (Hirai et al., 1981). Thirty minutes after the operation, 0.1 ml/kg of insulin preparation 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]. For the control studies, insulin was administered intravenously, intramuscularly, and subcutaneously to separate groups of animals. 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 stored at −18°C until analysis.

*Analytical method*

Plasma glucose level was estimated according to the *o*-toluidine method (Hyvärinen and Nikkilä, 1962). The decrement of the plasma glucose level (D, total decrease) from 0 to 4 h was calculated from the following equation:

$$D(\%) = \frac{AUC_c - AUC_s}{AUC_c} \times 100 \qquad (1)$$

where $AUC_c$ = area under the plasma glucose level versus time curve from 0 to 4 h after nasal administration of saline; and $AUC_s$ = area under the plasma glucose level versus time curve from 0 to 4 h after nasal administration of insulin.

## Results

Fig. 1 shows the change in plasma glucose levels after nasal administration of 10 U/kg of the insulin solution at pH levels 3.1, 5.5 and 7.4, and of 20 U/kg at pH 3.1. At both doses, administration of the pH 3.1 solution resulted in a dose-dependent decrease in the plasma glucose level: after 1 h the level had reached 70% (10 U/kg) and 45% (20 U/kg) of the initial value. The solutions at pH 5.5 and 7.4 produced little or no hypoglycemia.

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

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

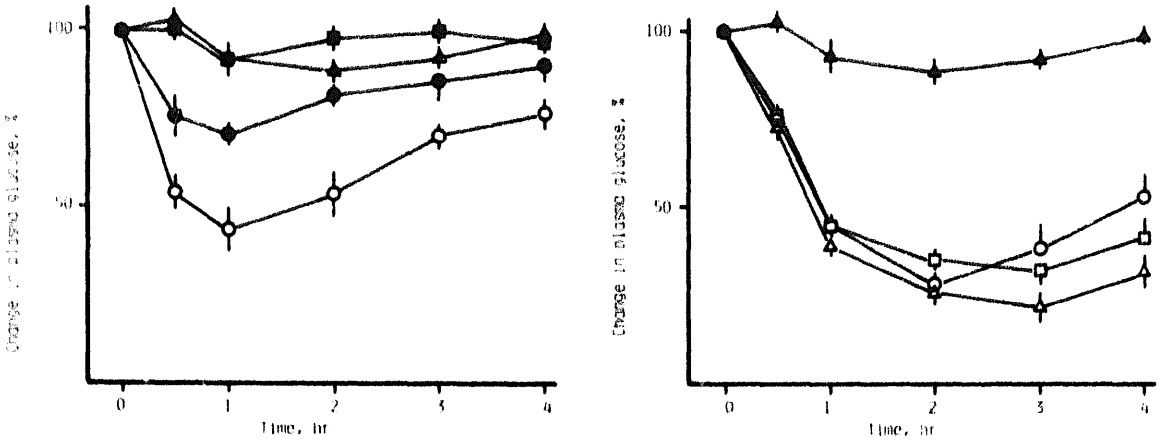


Fig. 1. Change in plasma glucose after nasal administration of insulin in rats. ●, pH 3.1 (10 U/kg); ○, pH 3.1 (20 U/kg); ■, pH 5.5 (10 U/kg); ▲, pH 7.4 (10 U/kg). The data are expressed as mean ± S.E. of 4 animals.

Fig. 2. Effects of surfactants on plasma glucose after nasal administration of insulin at a dose of 10 U/kg in rats (pH 7.4, 1% surfactant). ▲, no surfactant (n = 4); ○, sodium glycocholate (n = 5); □, saponin (n = 6); △, polyoxyethylene 9 lauryl ether (n = 9). The data are expressed as mean ± S.E.

When a surfactant, such as sodium glycocholate, saponin or polyoxyethylene-9-lauryl ether, was added at a concentration of 1% to the insulin solution at pH 7.4, the hypoglycemia was enhanced significantly in both extent and duration: the minimum glucose level, about 25% of the initial level, was observed 2 or 3 h after administration (Fig. 2).

Fig. 3 shows the effects of concentration of the surfactants. The decrements of the plasma glucose level (total decrease) from 0 to 4 h gradually increased as the concentration increased from 0 to 0.5% and then plateaued.

Tables 1 and 2 show the effects of the addition of 1% surfactants at pH 7.4 and a dose of 10 U/kg. The plasma glucose level was reduced by the addition of the non-ionic ether type, the anionic and the amphoteric surfactants. Bile acid salts, saponin and surfactin, a bacterial peptide lipid surfactant isolated from *Bacillus subtilis* (Arima et al., 1968), also decreased the plasma glucose level significantly. The non-ionic ester type surfactants were less effective than the ether type and the vehicles—propylene glycol and polyoxyethylene glycol 1 000—were ineffective.

To investigate in more detail the relation between the physicochemical property of the non-ionic surfactants and the observed promoting effect, the influence of the average number of ethylene oxide units in the polyoxyethylene moiety or the number of carbon atoms in the alcohol moiety was examined. Fig. 4 shows the plot of the decrement of plasma glucose level versus the HLB values of the non-ionic surfactant. The addition of a surfactant having a HLB value from 8 to 14 resulted in the greatest decrement of the plasma glucose level: the polyoxyethylene butyl ethers and the ester type surfactants were exceptions.

To estimate the bioavailability of the nasally administered insulin, the relation between dose and the hypoglycemic effect at pH 3.1 and pH 7.4 in the presence of

Table 1

Effects of non-ionic surfactants (1%) on the nasal absorption of insulin (10 U/kg) in rats

| Surfactants | HLB | Number of animals | D [a] (%) |
|---|---|---|---|
| No surfactant | | 4 | 6.0 ± 0.9 |
| Ether type: | | | |
| P.O.E. 5 [g] butyl ether [b] | 13.0 | 4 | 10.7 ± 4.0 |
| P.O.E. 10 butyl ether [b] | 16.8 | 4 | −0.4 ± 1.8 * |
| P.O.E. 20 butyl ether [b] | 20.7 | 4 | 3.1 ± 2.8 |
| P.O.E. 5 octyl ether [b] | 10.5 | 4 | 55.4 ± 4.8 ** |
| P.O.E. 10 octyl ether [b] | 13.9 | 4 | 51.6 ± 2.8 ** |
| P.O.E. 20 octyl ether [b] | 17.3 | 4 | 24.2 ± 2.5 ** |
| P.O.E. 5 lauryl ether [b] | 8.6 | 5 | 59.3 ± 3.0 ** |
| P.O.E. 9 lauryl ether [b] | 11.5 | 9 | 60.9 ± 2.9 ** |
| P.O.E. 10 lauryl ether [b] | 12.1 | 4 | 63.3 ± 2.5 ** |
| P.O.E. 20 lauryl ether [b] | 15.5 | 5 | 50.8 ± 2.3 ** |
| P.O.E. 5 cetyl ether [b] | 7.2 | 4 | 13.5 ± 3.6 |
| P.O.E. 10 cetyl ether [b] | 10.6 | 4 | 64.0 ± 1.3 ** |
| P.O.E. 20 cetyl ether [b] | 14.1 | 4 | 54.6 ± 2.4 ** |
| P.O.E. 5 stearyl ether [b] | 6.6 | 4 | 3.9 ± 4.0 |
| P.O.E. 10 stearyl ether [b] | 10.1 | 4 | 53.3 ± 2.9 ** |
| P.O.E. 20 stearyl ether [b] | 13.6 | 4 | 58.3 ± 3.4 ** |
| P.O.E. 10 nonylphenyl ether [b] | 11.7 | 4 | 57.3 ± 0.5 ** |
| P.O.E. 10 octylphenyl ether [b] | 12.3 | 4 | 55.7 ± 4.4 ** |
| P.O.E. 24 cholesteryl ether [c] | 14.0 | 4 | 54.4 ± 6.1 ** |
| Ester type: | | | |
| P.O.E. 10 monostearate [b] | 10.8 | 4 | 16.6 ± 3.2 * |
| P.O.E. 40 monostearate [b] | 17.4 | 4 | 5.5 ± 5.3 |
| P.O.E. 10 monolaurate [b] | 12.6 | 4 | 35.1 ± 6.9 ** |
| P.O.E. 20 sorbitan monooleate [d] | 14.4 | 4 | 6.4 ± 3.2 |
| P.O.E. 50 hydrogenated castor oil [e] | 13.4 | 4 | 10.3 ± 4.6 |
| Sucrose fatty acid ester [f] | 11.0 | 5 | 21.5 ± 2.1 ** |

[a] The decrement of plasma glucose level (D) was calculated from Eqn. 1 and expressed as mean ± S.E.
[b] Nihon Emulsion Co., Ltd., Tokyo, Japan.
[c] American Cholesterol Products Inc., New Jersey, U.S.A.
[d] Kao-Atlas Co., Ltd., Tokyo, Japan.
[e] Nikko Chemical, Ltd., Tokyo, Japan.
[f] Daiichi Kogyo Seiyaku Co., Ltd., Kyoto, Japan.
[g] The number of ethylene oxide units (P.O.E.) are denoted as P.O.E. n.
* $P < 0.05$ respect to no surfactant group.
** $P < 0.01$ respect to no surfactant group.

1% sodium glycocholate or polyoxyethylene-9-lauryl ether were investigated. The results, compared with those observed after intravenous, intramuscular or subcutaneous administration, are shown in Fig. 5. When the decrement of plasma glucose level from 0 to 4 h was plotted versus log dose of insulin, the curves for nasal and parenteral administration were parallel, although a shift to the right was observed for the nasal administration. About 20 times the dose of the pH 3.1 insulin solution given nasally without promoter was necessary to produce the equivalent hypo-

Table 2

Effects of various kinds of surfactants (1%) on the nasal absorption of insulin (10 U/kg) in rats

| Surfactants | Number of animals | D [a] (%) |
|---|---|---|
| Anionic: | | |
| Sodium laurylsulfate [b] | 4 | 52.9 ± 1.4 ** |
| Potassium laurate [b] | 4 | 55.2 ± 1.4 ** |
| Amphoteric: | | |
| Miranol C2M CONC [c] | 4 | 56.9 ± 2.5 ** |
| Bile acid salt: | | |
| Sodium taurocholate [d] | 4 | 56.7 ± 3.3 ** |
| Sodium cholate [d] | 4 | 53.1 ± 4.8 ** |
| Sodium deoxycholate [d] | 4 | 55.7 ± 6.3 ** |
| Sodium glycocholate [d] | 5 | 53.1 ± 2.7 ** |
| Sodium chenodeoxycholate [d] | 5 | 49.3 ± 1.0 ** |
| Glycoside: | | |
| Saponin [b] | 6 | 53.9 ± 2.5 ** |
| Peptidelipid: | | |
| Surfactin [e] | 4 | 54.1 ± 6.2 ** |
| Vehicle: | | |
| Propylene glycol [b] | 4 | 4.4 ± 6.8 |
| Polyethylene glycol 1000 [b] | 4 | 3.8 ± 2.0 |

[a] The decrement of plasma glucose level (D) was calculated from Eqn. 1 and expressed as mean ± S.E.
[b] Wako Pure Chemical Industries, Ltd., Osaka, Japan.
[c] Miranol Chemical Co., Inc., New Jersey, U.S.A.
[d] Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan.
[e] Takeda Chemical Industries, Ltd., Osaka, Japan.
** $P<0.01$ respect to no surfactant group.

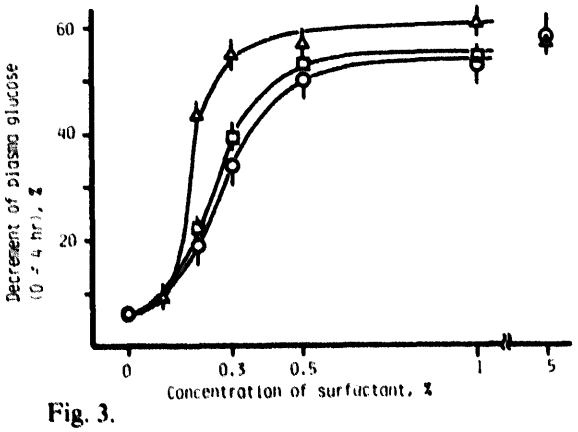


Fig. 3.

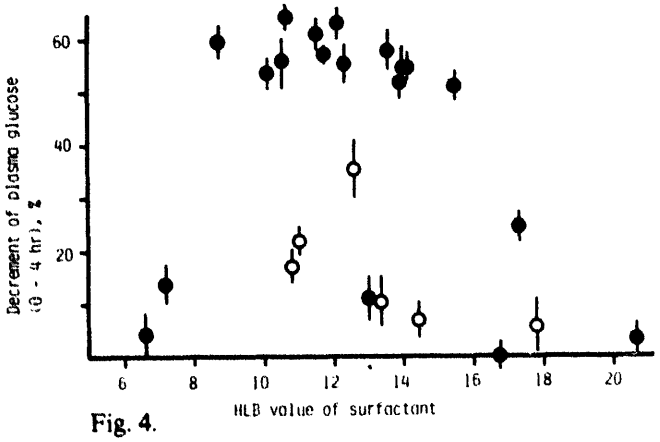


Fig. 4.

Fig. 3. Effects of concentrations of surfactants on the nasal absorption of insulin (10 U/kg) as reflected by the decrement of plasma glucose in rats. ○, sodium glycocholate; □, saponin; △, polyoxyethylene 9 lauryl ether. The data are expressed as mean ± S.E. (n=4–9).

Fig. 4. Relation between HLB values of non-ionic surfactants and the nasal absorption of insulin (10 U/kg) in rats. ●, ether type surfactant; ○, ester type surfactant. The data are expressed as mean ± S.E. (n=4–9).

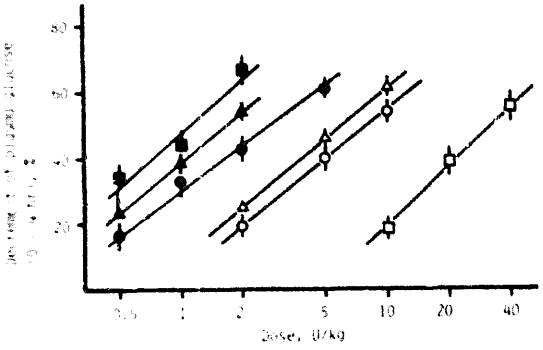

Fig. 5. Dose–response curves after nasal, intravenous, subcutaneous and intramuscular administration of insulin in rats. □, nasal (no surfactant); ○, nasal (1% sodium glycocholate); △, nasal (1% polyoxyethylene-9-lauryl ether); ●, intravenous; ▲, subcutaneous; ■, intramuscular. The data are expressed as mean ± S.E. (n = 4–9).

glycemic effect observed after intravenous administration. However, only 3 times the dose of the pH 7.4 insulin solution with promoters was enough to produce the equivalent hypoglycemic effect observed after intravenous administration.

## Discussion

On the basis of our previous reports, it was evident that, in dogs and humans, insulin was easily absorbed from the nasal cavity into the systemic blood circulation in an active form without being subjected to metabolism (Hirai et al., 1978; Yokosuka et al., 1977). In the dog study, the nasal absorption of insulin was enhanced when it was dissolved in an acid medium: the bioavailability of this formulation was about 25% compared with the hypoglycemic effect following intravenous administration. However, in the human study, the bioavailability of the acid insulin preparation was not sufficient and to enhance the nasal absorption, the addition of a promoter, such as sodium glycocholate, was necessary.

The results of the present study indicate that the absorption of insulin through the nasal mucosa in rats shows the same tendency observed in humans. The absolute bioavailability of insulin estimated by the hypoglycemic effect was 5% after nasal administration of the pH 3.1 insulin solution without promoter. However, the bioavailability was increased to about 30% by the addition of a promoter, such as sodium glycocholate or polyoxyethylene-9-lauryl ether, at a concentration of 1%.

Various kinds of surfactants showed this absorption promotion. They include non-ionic ether type, anionic, amphoteric surfactants, and the bile acid salts, saponin and surfactin. Among the non-ionic surfactants, the addition of one having a HLB value from 8 to 14 was found to produce the greatest promoting effect.

It is well known that surfactants can influence drug absorption and membrane transport. However, the mode of this influence has been shown to be highly variable. For example, Levy et al. (1968, 1966) and Kakemi et al. (1965) have shown that the gastrointestinal absorption rate of secobarbital or sulfisoxazole was increased signifi-

cantly in the presence of low concentrations of polysorbate 80 and was decreased under high concentrations of the surfactant. These results indicate that low concentrations of the surfactant enhance the absorption of such a lipophilic drug by increasing the solubility of the drug and the permeability of biological membranes to the drug. At significantly high concentrations of the surfactant, above the critical micelle concentration, the absorption rate depends on the thermodynamic activity of the drug which decreases with increasing surfactant concentration.

However, a number of reports concluded that non-ionic, anionic surfactants, and bile acids enhanced the gastrointestinal absorption of water-soluble, micelle-free drugs, such as parenteral antibiotics (Davis et al., 1970), vitamin $B_{12}$ (Davis and Kreutler, 1971), phenol red (Khalafallah et al., 1975; Gouda et al., 1977), thiamine disulfide compounds (Utsumi et al., 1974) and erythritol (Birkett and Silen, 1974), by increasing the mucosal permeability. Moreover, Ichikawa et al. (1980) and Nishioka and Kawamura (1977) have shown that the rectal absorption of insulin was enhanced in the presence of non-ionic surfactants or bile acids.

The results of the present study suggest that the surfactants appear to increase the permeability of the nasal mucosa, and thereby promote the nasal absorption of a high-molecular weight polypeptide such as insulin. Another possible explanation is that the surfactants reduced the proteolytic enzyme activity in the nasal mucosa. Detailed mechanisms of the promoting effect of the surfactants on the nasal absorption of insulin will be presented in a subsequent paper.

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