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Rectal absorption of insulin suppositories in rabbits

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When insulin solutions (100 U kg^{-1}) at various pH values were placed in the rectum of rabbits, a large decrease in blood glucose concentration was observed except at the pH close to the iso-electric point of insulin. The effect of surface-active agents on the rectal absorption of insulin was examined by measuring the blood glucose concentration after the administration of 2 or 5 U kg^{-1}. Non-ionic ether type, anionic, cationic and amphoteric surfactants as well as bile acids increased the absorption. The optimal effect with suppositories was reached with the addition of 1% polyoxyethylene (9) lauryl alcohol ether. Insulin suppositories containing agents enhancing rectal absorption were compared with the insulin for intravenous, intramuscular and subcutaneous injection. The changes of blood glucose and plasma immuno-reactive insulin concentration after rectal administration of insulin were similar to those after intravenous injection.

The rectal absorption of insulin has been described by Matsubara (1963), Nishioka & Kawamura (1977), Shichiri et al (1978) and Touitou et al (1978). But in those studies, the fall of concentration of blood glucose was observed only with a large dose of insulin. Therefore, we have attempted to improve the absorption of insulin by the addition of surfactants and to obtain a satisfactory fall of blood glucose concentration with a lower dose of insulin.

MATERIALS AND METHODS

Materials

Bovine crystalline insulin (Sigma Chem. Co.) with 24 U mg^{-1} contained 0·5% Zinc. Corn oil (Japan Maize Ind. Co.) was used as the suppository base. Surfactants, bile acids and phospholipid used are listed in Table 1 and 2.

Preparations

Insulin solutions of various pH values were prepared by dissolving or suspending crystalline insulin in 0·1 M citrate buffer or 0·1 M phosphate buffer. Insulin suppositories were prepared by mixing insulin and various concentrations of surfactants or bile acids in corn oil. Insulin injection (0·5 U/0·25 ml kg^{-1}) was prepared by dissolving 200 U of crystalline insulin in 100 ml of 1·4% (w/v) glycerol 0·1% (w/v) phenol and 0·01 M hydrochloric acid.

Methods

White male rabbits, 2·0 to 2·4 kg, fasted for 24 h to render the rectum free of excess faecal matter, were constrained for least for 3 h to avoid stress effects before administration of the suppository. A glass injector (tube length of 7·5 cm, outer diameter 1·0 cm) containing 1·0 g of insulin suppository was inserted into the rectum about 3·0 cm deep from the anus and the suppository ejected. To prevent expulsion the distal end of the rectum was ligatured. Insulin injections (0·5 U/0·25 ml kg^{-1}) were administered intramuscularly and subcutaneously into the thigh or intravenously into the ear vein. After the administration, approximately 1·0 ml of blood was collected from the marginal ear vein into a heparinized tube at the indicated time interval. The blood glucose was measured by a glucose oxidase method (Schmidt 1963). Plasma immuno-reactive insulin (I.R.I.) was measured by a radio-immunoassay method (Morgan & Lazarow 1963). Each animal was used once.

RESULTS

Blood glucose concentrations after insulin (100 U kg^{-1}) at different pH values showed falls that increased in the order; pH 3 > pH 8 > pH 7 > pH 5 (Fig. 1). The solution of pH 3 caused hypoglycaemic convulsions.

Table 1 shows the effects of 3% surfactants in insulin suppositories (5 U kg^{-1}) in corn oil on the blood glucose concentration. Since lauroyl dimethylaminoacetic acid betaine was commercially available as a 26% aqueous solution, the insulin suppository was administered as a 3% emulsion of this surfactant. The blood glucose concentration was reduced by the addition of the non-ionic ether type, anionic, cationic and amphoteric surfactants. In particular, polyoxyethylene (9) lauryl alcohol ether, polyoxy-

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Table 1. Effects of surfactants in insulin suppositories on blood glucose concentration in rabbits. Insulin suppositories contained 3% surfactants, 5 U kg^{-1} of insulin and corn oil. The initial blood glucose concentration was 131·4 ± 5·0 mg/100 ml. Each value represents the blood glucose concentration at 30, 60, 90 and 120 min after rectal administration of insulin suppositories and the mean of 3 rabbits.

Surfactant and HLB		Decrease in blood glucose % 30	60	90	120 (min)
Control: P.O.E. (9) lauryl alcohol ether[a]	HLB = 11·5				
without surfactant		+2·7	−0·5	+1·3	−1·5
without insulin		+3·3	−1·1	+2·8	+3·4
Non-ionic:					
P.O.E. (9) lauryl alcohol ether	HLB = 11·5	−32·5	−58·8	−50·8	−40·3
P.O.E. (10) nonylphenyl ether[a]	HLB = 16·5	−28·9	−54·8	−54·5	−51·1
$HO(CH_2CH_2O)_a(CH_2CH_2CH_2O)_b$-$(CH_2CH_2O)_c$ H[c]		−14·3	−25·2	−22·7	−7·5
P.O.P. (4) P.O.E. (10) cetyl ether[a]	HLB = 10·6	−24·6	−25·8	−28·6	−16·1
Glyceryl monostearate[a]	HLB = 4·5	+15·8	+17·8	+29·4	+18·0
P.O.E. (20) sorbitan monooleate[a]	HLB = 15	+3·6	+9·1	+0·1	−2·1
Sucrose fatty acid ester[d]	HLB = 11	+4·4	+9·6	+10·6	+16·2
P.O.E. (30) lanolin derivative[a]	HLB = 14·2	+15·1	+11·7	+17·4	+14·0
P.O.E. (60) caster wax derivative[a]	HLB = 14·1	+29·9	+36·0	+30·7	+28·3
P.O.E. (15) monooleate[a]	HLB = 13·4	−3·4	−8·3	+2·3	0
Anionic:					
Sodium lauryl sulfate[a]		−16·2	−48·8	−58·2	−53·7
Sodium P.O.E. (2) lauryl ether sulfate[a]		−21·4	−37·7	−27·4	−4·5
Sodium di P.O.E. (10) lauryl ether phosphate[a]		−3·4	−30·0	−10·0	−9·5
Monosodium *N*-lauroyl L-glutamate[e]		−42·1	−54·4	−61·4	−57·8
Sodium *N*-lauroyl-sarcosinate[a]		−31·5	−35·9	−48·6	−43·7
Sodium di-2 ethylhexyl sulfosuccinate[a]		−17·5	−44·8	−48·8	−45·5
Cationic:					
Stearyl trimethyl ammonium chloride[b]		−10·6	−32·7	−40·9	−47·1
P.O.E. (5) oleyl amine[a]		−18·3	−49·0	−58·2	−56·7
P.O.E. (5) oleyl amide[a]		−40·3	−45·5	−35·8	−23·8
Amphoteric:					
Lauroyl dimethyl amino acetic acid betaine[b]		−24·6	−35·4	−42·9	−34·3

Supplied by [a] Nikko Chem. Co. Ltd. [b] Kao-Atlas Co. Ltd. [c] Asahi Denka Kogyo Co. Ltd. [d] Daiichi Kogyo Seiyaku Co. Ltd. and [e] Ajinomoto Co. Ltd.

Table 2. Effects of bile acids and phospholipid in insulin suppositories on blood glucose in rabbits. Insulin suppositories contained 2% bile acid, 2 U kg^{-1} of insulin and corn oil. When egg lecithin was used, a dose of insulin was 5 U kg^{-1}. The initial blood glucose concentration was 131·4 ± 5·0 mg/100 ml. Each value represents the blood glucose at 30, 60, 90 and 120 min after rectal administration of insulin suppositories and the mean of 3 rabbits.

Bile acids and phospholipid	Decrease in blood glucose % 30	60	90	120 (min)
Bile acid:				
Cholic acid[a]	−10·6	−53·4	−52·9	−46·7
Sodium cholate[a]	−23·2	−47·8	−37·7	−21·4
Sodium taurocholate[b]	−39·9	−48·5	−29·3	−17·4
Glycocholic acid[a]	−44·0	−56·2	−49·2	−31·6
Chenodeoxycholic acid[a]	−29·7	−53·5	−46·7	−32·3
Phospholipid:				
Egg lecithin[a]	−9·2	−13·7	−23·6	−15·9

Supplied by [a] Sigma Chem. Co. and [b] Calbiochem.

ethylene (10) nonylphenyl ether, sodium lauryl sulphate, monosodium *N*-lauroyl 6-glutamate and polyoxyethylene (5) oleylamine markedly decreased the blood glucose concentration. Some of the non-ionic surfactants of the ester type effected a smaller decrease in blood glucose than those of ether type, other ester type surfactants were ineffective.

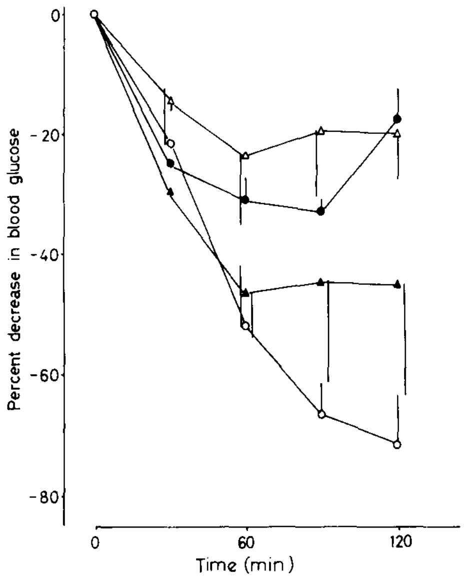

FIG. 1. Effects of different pH values on blood glucose concentration after rectal administration of insulin in rabbits. Insulin suppositories (100 U kg⁻¹) were prepared with pH 3 (○) and pH 5 (▲) in 0·1 M citrate buffer, pH 7 (●) and pH 8 (▲) in 0·1 M phosphate buffer. The initial blood glucose concentration was 131·0 ± 7·7 mg/100 ml. Each value represents the blood glucose concentration at 30, 60, 90 and 120 min after rectal administration of insulin suppositories and the mean of 3 rabbits ± s.e.m.

Table 2 shows the effects on blood glucose of 2% bile acids with 2 U kg^{-1} insulin, and egg lecithin with 5 U kg^{-1} insulin in corn oil after administration as suppositories. Egg lecithin was less effective than bile acids.

Since the presence of polyoxyethylene (9) lauryl alcohol ether in insulin suppositories resulted in a marked decrease in blood glucose concentration (Table 1), the influence of the average number of ethylene oxide residues in the polyoxyethylene moiety and number of carbon atoms in the alcohol moiety was examined on the blood glucose concentration. The decrease was greatest when the average of the ethylene oxide residue was 9 (Table 3). With the alcohol moiety, lauryl alcohol with 12 carbon atoms showed the greatest effect. The result indicated polyoxyethylene (9) lauryl alcohol ether to be among the most effective of polyoxyethylene fatty alcohol ethers in decreasing blood glucose concentration.

The concentration that exerted optimal effect in suppositories was 1·0% (Fig. 2).

Suppositories containing 1% polyoxyethylene (9) lauryl alcohol ether with all doses of insulin gave a peak time for the decrease in blood glucose concentration at 60 min after insertion. When 0·5 U kg^{-1} insulin suppositories were inserted, the blood glucose concentration was decreased by 37% of the initial value and the plasma I.R.I. value rose to a maximum of 123 μU ml^{-1} at 15 min. Insulin suppositories caused a large fall of blood glucose and a longer hypoglycaemia with increasing dose of insulin (Fig. 3).

A comparison of insulin suppositories (containing 1·0 U kg^{-1} of insulin and 1% polyoxyethylene (9)

Table 3. Effects of polyoxyethylene (P.O.E.)(*n*) fatty alcohol ethers in insulin suppositories on blood glucose level in rabbits. Insulin suppositories contained 0·5% polyoxyethylene(n) fatty alcohol ethers and 1 U kg^{-1} of insulin in corn oil. The initial blood glucose concentration was 118·3 ± 6·2 mg/100 ml. Each value represents the blood glucose concentration at 30, 60, 90 and 120 min after rectal administration of insulin suppositories and mean of 3 rabbits ± s.e.m.

Surfactants P.O.E.(n)—alcohol ethers	Decrease in blood glucose % 30	60	90	120 (min)
(3) lauryl	−8·8 ± 7·7	−3·0 ± 3·5	−11·2 ± 2·3	−9·8 ± 4·6
(6) lauryl	−12·3 ± 0·6	−23·8 ± 6·9	−20·6 ± 6·5	−11·2 ± 6·1
(9) lauryl	−12·7 ± 8·5	−47·9 ± 5·6	−47·1 ± 7·4	−32·6 ± 11·2
(25) lauryl	+0·6 ± 1·2	−4·2 ± 2·6	−4·0 ± 2·8	−0·9 ± 1·0
(40) lauryl	+17·3 ± 2·9	+18·5 ± 2·5	+14·9 ± 3·1	+13·5 ± 8·2
(9) octyl	+3·9 ± 5·5	+12·8 ± 8·0	+13·6 ± 9·2	+13·0 ± 6·2
(9) decanyl	−21·6 ± 4·8	−36·2 ± 3·7	−16·2 ± 5·1	−12·6 ± 6·8
(9) cetyl	−28·4 ± 3·6	−43·1 ± 2·6	−35·9 ± 5·7	−16·8 ± 5·4
(9) stearyl	−22·0 ± 6·2	−22·2 ± 3·2	−19·8 ± 4·8	−26·2 ± 7·9

All supplied by Nikko Chem. Co. Ltd.

lauryl alcohol ether in corn oil) with insulin solution for intravenous, intramuscular and subcutaneous injection (containing 0·5 U kg^{-1} of insulin) showed the changes in blood glucose and plasma I.R.I. concentration after rectal administration to be similar to those after intravenous injection (Fig. 4).

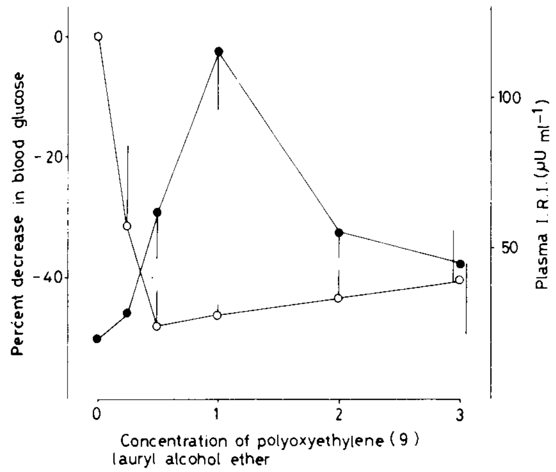

FIG. 2. Effects of concentration of polyoxyethylene (9) lauryl alcohol ether in insulin suppositories on blood glucose and plasma I.R.I. concentration in rabbits. Insulin suppositories contained 1 U kg^{-1} of insulin and indicated concentrations of polyoxyethylene (9) lauryl alcohol ether in corn oil. The initial blood glucose concentration was 117·5 ± 5·6 mg/100 ml. Each value represents the blood glucose (○) at 60 min and plasma I.R.I. (●) at 30 min after rectal administration of insulin suppositories and the mean of 5 rabbits ± s.e.m.

DISCUSSION

In the present experiments on the enhancement of rectal absorption of insulin by surfactants, the changes in blood glucose concentration were influenced by all surfactants tested. The ability of bile acids approached that of the synthetic surfactants while egg lecithin was weaker.

Rectal absorption of insulin was affected by both the average number of the ethylene oxide residue and the chain length of the alcohol moiety. Polyoxyethylene (9) lauryl alcohol ether was the most effective in promoting rectal absorption at a concentration in suppositories of 1%.

On the rectal absorption of insulin suppositories with surface-active agents, Matsubara (1963) reported that, when the insulin suppositories with Emanone 4115 (polyoxyethylene monooleate) were rectally administered to rabbits at dose of 30 U kg^{-1}, the decreases in the blood glucose concentration

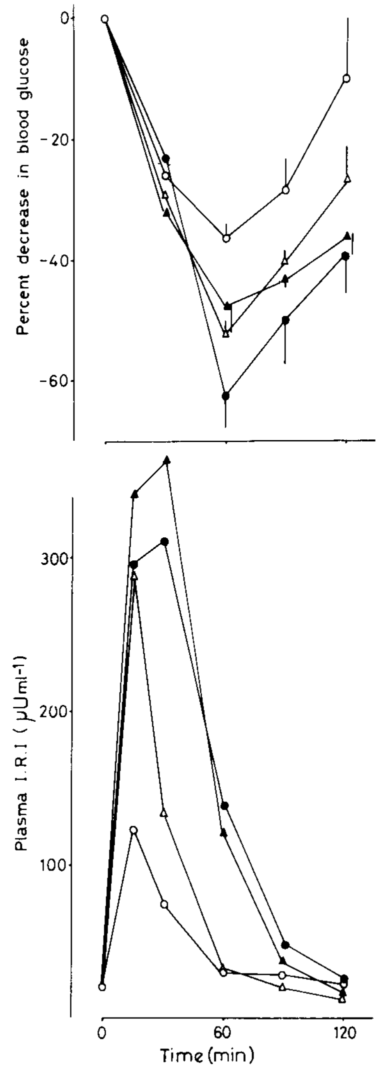

FIG. 3. Effects of varying doses of insulin suppositories on blood glucose and plasma I.R.I. concentration in rabbits. Insulin suppositories contained 1·0% polyoxyethylene (9) lauryl alcohol ether and 0·5 (○), 1·0 (△), 2·0 (●) and 3·0 U kg^{-1} (▲) of insulin in corn oil. The initial blood glucose concentration was 101·5 ± 4·8 mg/100 ml. Each value represents the blood glucose and plasma I.R.I. level after rectal administration of insulin suppositories and the mean of 5 rabbits ± s.e.m.

were 30 to 40 mg/100 ml. Nishioka & Kawamura (1977) reported that the rectal administration of 100 U kg^{-1} of insulin suppositories with HCO-60 (polyoxyethylene (60) caster oil derivative) to rabbits caused 40% decrease in the blood glucose concen-

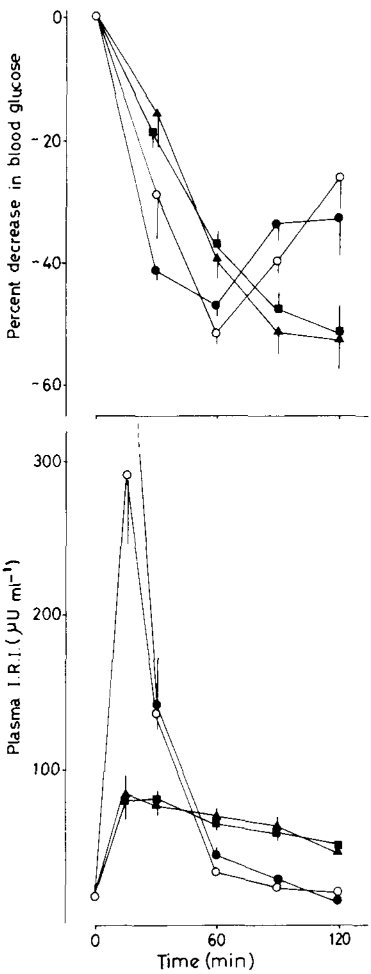

FIG. 4. Effects of route of administration on blood glucose and plasma I.R.I. concentration in rabbits. Insulin suppositories (○) contained 1 U kg^{-1} and 1·0% polyoxyethylene (9) lauryl alcohol ether in corn oil. Insulin injection (●: intravenous ▲: intramuscular and ■: subcutaneous) contained 0·5 U kg^{-1} of insulin in insulin solution. The initial blood glucose concentration was 113·5 ± 8·5 mg/100 ml. Each value represents the blood glucose and plasma I.R.I. concentration after the administration of insulin suppositories and insulin injection, and the mean of 5 rabbits ± s.e.m.

tration. The rectal administration of 1 U kg^{-1} insulin suppositories with 1% polyoxyethylene (9) lauryl alcohol ether in corn oil caused about 50% decrease of the initial value. Thus, the hypoglycaemic effect produced by the insulin suppositories we tested was caused by smaller dose of insulin than that used by Matsubara (1963) or Nishioka & Kawamura (1977). The dose of insulin in suppositories needed to be about two or three times the intravenous dose to produce hypoglycaemia of similar magnitude.

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