STUDIES ON THE PROMOTING EFFECTS OF MEDIUM CHAIN FATTY ACID SALTS ON THE NASAL ABSORPTION OF INSULIN IN RATS

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We investigated the promoting effect of medium chain fatty acid salts such as sodium caprylate, sodium caprate (Na C10) and sodium laurate on rat nasal absorption of insulin. Further, we studied the possible mechanism of the absorption promoting effect by fatty acid salts in terms of hemolytic activity, osmotic pressure, chelating effect and inhibition of leucine aminopeptidase activity. Na C10 exhibited the strongest absorption promoting effect on insulin among these fatty acid salts. The promoting effect of Na C10 was very rapid reaching the maximum immunoreactive insulin level within 5 min and its optimum concentration was about 1%. The amount of insulin absorbed after a nasal administration with Na C10 was about 98% of one half subcutaneous dose. The absorption promoting effect of fatty acid salts on insulin was at least in part associated with the chelating ability for calcium ions and the inhibitory action on leucine aminopeptidase activity.

Keywords — insulin; nasal absorption; medium chain fatty acid salts; sodium caprylate; sodium caprate; sodium laurate; hemolytic activity; osmotic pressure; leucine aminopeptidase; chelating ability

INTRODUCTION

Insulin is an indispensable pancreatic peptide hormone for insulin-dependent diabetic patients. It is well known that the hormone is poorly absorbed after oral administration because of its degradation by gastrointestinal proteases and its poor permeability through the gastrointestinal mucosa due to its high molecular weight. Therefore, the hormone is administered only by intravenous, subcutaneous or intramuscular injection. However, patients must endure pain from injection and are occasionally exposed to hypertrophy or atrophy of subcutaneous fat at injection sites from long-term administration.

Recently, many investigators have reported that the bioavailability of poorly absorbed drugs and peptides through rectal or nasal mucosa was enhanced by various absorption promoters such as bile salts,¹⁻⁵⁾ surfactants,^{1,6)} enamine derivatives of amino acids,⁷⁻⁹⁾ glycerin esters,^{10,11)} salicylates¹²⁻¹⁴⁾ and carboxylic acids.¹⁵⁾ In particular, rectal absorption of β -lactam antibiotics which are poorly absorbed was enhanced by medium chain carboxylic acids,¹⁵⁾ but the promoting effect of carboxylic acids on nasal mucosa has not been reported. In our previous paper, we reported on nasal administration of glucagon aerosol with sodium caprate (Na C10)

in a totally pancreatectomized patient. The hyperglycemic effect after the nasal administration was the same as that after the subcutaneous injection.¹⁶⁾

In the present study, we investigated the promoting effect of medium chain fatty acid sodium salts on nasal absorption of insulin in rats.

MATERIALS AND METHODS

Materials — Sodium caprylate (Na C8), sodium caprate (Na C10) and sodium laurate (Na C12) were obtained from Tokyo Kasei Kogyo Co. Sodium glycocholate was obtained from Nakarai Chemical Co. Insulin (23.4 I.U./mg) was obtained from Sigma Chemical Co. Ethylenediaminetetraacetic acid disodium salt (EDTA·2Na), “Insulin C-Test Wako” and “Glucose B-Test Wako” were obtained from Wako Pure Chemical Industries.

Preparation — Ten μ l of HCl were added to an insulin (500 U) suspension in 300 μ l of distilled water to dissolve the drug and then the solution was neutralized with sodium hydroxide. This solution was diluted to contain 100 U/ml of insulin either by the addition of 0.01 M phosphate buffer solution, pH 7.4 or by buffers containing each promoter.

Animal Experiments — Male Wistar rats,

200–300 g, were fasted overnight before experiments and were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg during entire intravenous, subcutaneous and nasal absorption studies. For the nasal absorption studies, the surgical operation was made according to the method of Hussain *et al.*¹⁷⁾ and insulin solution was administered (0.1 ml/kg) to rats. For intravenous administration, insulin (5 U/kg) was administered by bolus injection into the femoral vein. For subcutaneous administration, insulin (5 U/kg) was injected under the abdominal skin. One half ml of blood was taken periodically from the femoral aorta by a syringe containing heparin (2.5 U) saline solution and plasma was separated by centrifugation at 3000 rpm for 10 min. Samples were stored at -20°C until analysis.

Analytical Method — Plasma immunoreactive insulin (IRI) levels were determined by an enzyme immunoassay kit by use of the *o*-phenylene-diamine method. Plasma glucose levels were determined by the mutarotase/glucose oxidase method. A decrement of the plasma glucose level among each sampling time interval was calculated by a modification of the method of Hirai *et al.*¹⁾ That is to say, the decrement ($D\%$) was calculated by the following equation;

$$D(\%) = \{(AUC_c - AUC_s)/AUC_c\} \times 100$$

where AUC_c is the area under the plasma glucose level *versus* time curve after each sampling time following nasal administration of phosphate buffer and where AUC_s is the area under the plasma glucose level *versus* time curve after each sampling time following nasal administration of insulin or insulin with fatty acid salts.

Erythrocyte Hemolysis — Erythrocyte hemolysis experiments were performed by the method of Hirai *et al.*¹⁸⁾ Human nonfibrinated blood was obtained by centrifugation at 2000 rpm for 5 min. After removing the supernatant solution, the erythrocytes were washed 3 times in 3 ml of the physiological saline. The washed erythrocytes were suspended in 50 ml of the saline. Test solutions of medium chain fatty acid salts or Na glycocholate were prepared by dissolving them in the saline at various concentrations. Five ml of the test solution was added to 0.2 ml of the erythrocyte suspension. After incubation for 5 min at 25°C , the mixture was centrifuged for 5 min

at 2000 rpm, and then the absorbance of the supernatant at 540 nm was measured to calculate percentage of hemolysis.

Measurement of Osmotic Pressure — The osmotic pressure of the above test solution was measured by the freezing point lowering method with a Fiske OS[®] osmometer (Fiske Associates, Massachusetts).

Chelating Ability of the Fatty Acid Salts — Chelating ability of the promoters was determined for calcium ion by a modification of the method of Yata *et al.*¹⁹⁾ and was measured by a coloring method using Eriochrome[®] black T. A small amount of Eriochrome[®] black T powder was added to 0.25 ml of 1/200 M CaCl_2 –1/150 M NH_4OH – NH_4Cl buffer, pH 10.0, and then the solution was titrated with the promoter solution until its color was changed from red to blue. In order to distinguish blue coloration more clearly, the promoter solution was prepared by dissolving Na C8, Na C10 or Na C12 in the buffer, which were made up to the final concentration of 4%, 0.5% or 1%, respectively.

Determination of Leucine Aminopeptidase Activity in the Nasal Mucosa Homogenate — After the rats or rabbits were anesthetized with ether and decapitated, the nasal mucosa on the septal cartilage was isolated from the frontal bone and was homogenized in a 10-fold volume of the cold saline with Phycotron[®]. Fatty acid salts or Na glycocholate solutions were prepared by dissolving them in the 0.05 M phosphate buffer, pH 7.0, at various concentrations. Determination of leucine aminopeptidase activity was made according to the method of Hirai *et al.*¹⁸⁾ and absorbance of the mixture was measured at 450 nm.

RESULTS

Figure 1 shows the IRI levels and plasma glucose levels in rats after nasal administration of 10 U/kg insulin solution.

Control plasma IRI levels in rats were maintained at about $10\ \mu\text{U/ml}$ throughout the experimental period. Plasma IRI levels were increased to about $40\ \mu\text{U/ml}$ after nasal administration of 10 U/kg insulin. On the other hand, plasma glucose levels following nasal administration of the buffer solution and 10 U/kg insulin solution were hardly different.

Figure 2 shows plasma IRI levels and relative decrement of plasma glucose in rats after nasal

FIG. 1. Change in Plasma Immunoreactive Insulin Levels ($\circ$, Insulin Alone; $\square$, Buffer Alone) and Plasma Glucose Levels ($\bullet$, Insulin Alone; $\blacksquare$, Buffer Alone) after Nasal Administration of Insulin (10 U/kg) in Rats

Each point represents the mean $\pm$ S.E.M. of 4 or 6 animals.

administration of various doses of insulin with 1% Na C10.

The absorption of insulin through the nasal mucosa was very rapid and plasma IRI levels de-

creased to near the control level about 1 h after the nasal administration of insulin at a dose of 2 and 5 U/kg. Plasma glucose levels reached minimum about 1 h after the nasal administration of each insulin dose.

The AUC_{0-4} of plasma IRI and relative decrement of plasma glucose after intranasal administration of insulin with one of the three fatty acid sodium salts or sodium glycocholate in rats are shown in Table I.

We compared the amount of insulin absorbed after nasal administration with that after subcutaneous injection because diabetic patients were treated usually by subcutaneous injection. The AUC_{0-4} value of plasma IRI after nasal administration of 10 U/kg insulin alone was $172 \text{ h} \cdot \mu\text{U/ml}$ and its relative absorption was 10% of that after subcutaneous injection. The AUC_{0-4} values of plasma IRI levels at an insulin dose of 10, 5 and 2 U/kg with sodium caprate (Na C10) were 1419, 200 and $174 \text{ h} \cdot \mu\text{U/ml}$, respectively. These results indicated that absorption of insulin was increased with dose in a non-linear manner when administered nasally with Na C10. The AUC_{0-4} values of plasma IRI after nasal administration of 10 U/kg insulin with sodium caprylate (Na C8) or sodium laurate (Na C12) are also shown in Table I. With respect to the effect of

FIG. 2. Change in Plasma IRI (A) and Glucose (B) Levels in Rats after Intranasal Administration of Insulin at a Dose of 2 ($\square$), 5 (Δ) and 10 U/kg ($\circ$) with 1% Na Caprate

Each point represents the mean $\pm$ S.E.M. of 3 animals.

TABLE I. Mean Area Under Plasma Immunoreactive Insulin Level-Time Curve after Intranasal Administration of Insulin With Promoters in Rats

Promoter	Dose U/kg	$AUC_{0-4}^a)$ $h \cdot \mu U/ml$	Relative absorption (%) ^{b)}	$D^c) (\%)^a)$
None (control)	0	28.7 ± 5.1	0	0
Nasal				
None	10	172 ± 14.2	10.1	3.9 ± 11.0
Na caprylate	10	410 ± 42.8	26.8	60.2 ± 4.2
Na caprate	10	1419 ± 94.2	97.6	65.3 ± 1.1
	5	200 ± 18.5	12.1	57.1 ± 6.4
	2	174 ± 7.6	10.2	23.4 ± 7.1
Na laurate	10	807 ± 122	54.6	82.5 ± 1.7
Na glycocholate	10	1021 ± 118	69.7	74.4 ± 3.4
	5	531 ± 19.5	35.3	64.3 ± 3.4
	2	262 ± 35.4	16.4	61.1 ± 6.7
	1	166 ± 11.5	9.7	16.0 ± 15.6
i.v.	5	3576 ± 507	249	81.8 ± 1.0
s.c.	5	1453 ± 260	100	76.2 ± 4.4

a) Each value is the mean $\pm$ S.E.M. for 3 or 6 animals.

b) Relative absorption: $\frac{AUC_{(nasal)} - AUC_{(control)}}{AUC_{(s.c.)} - AUC_{(control)}} \times 100$

c) D is relative decrement of plasma glucose levels.

carbon chain length of fatty acids on nasal absorption of insulin in rats, the AUC_{0-4} values of insulin exhibited a maximum with Na C10. Therefore, the best absorption promoting effect was exhibited by Na C10.

FIG. 3. Effect of Na Caprate Concentration on Nasal Absorption of Insulin (5 U/kg) in Rats
Each point represents the mean $\pm$ S.E.M. of 3 or 6 animals.

The AUC_{0-4} values of plasma IRI levels at insulin doses of 10, 5, 2 and 1 U/kg with sodium glycocholate were 1021, 531, 262 and 166 $h \cdot \mu U/ml$, respectively. These results indicated that the amount of insulin absorbed was roughly proportional to its dose in the presence of sodium glycocholate.

AUC_{0-4} values after intranasal administration were approximately 98% in the presence of Na C10 and 70% in the presence of sodium glycocholate compared with AUC_{0-4} following one half subcutaneous dose, and the relative decrement of plasma levels were 65% in Na C10 and 74% in sodium glycocholate. If insulin receptors are saturated by excessive insulin in blood, the hypoglycemic effect may reach the maximum value. Therefore, it seems that plasma IRI levels are not directly related to hypoglycemic effects. However, plasma glucose level tended to decrease to about 50% of control level about 50% of control level when the AUC_{0-4} value of insulin reached over 200 $h \cdot \mu U/ml$.

The absorbed insulin was found to be eliminated during the first 1 h after nasal administration of insulin at a dose of 5 U/kg with 1% Na C10 in rats, Figure 3 shows the effect of Na C10 concentration on nasal absorption of insulin in

FIG. 4. *Plasma Immunoreactive Insulin Profile after Nasal Administration of Insulin (5 U/kg) with 1% Na Caprylate in Rats*
Each point represents the mean $\pm$ S.E.M. of 3 animals.

rats by plotting AUC_{0-1} against promoter concentrations.

At 0.1 and 0.5% Na C10, these AUC_{0-1} values were 34.56 and 52.50 h $\cdot$ μ U/ml, respectively. But at 1% and 2% Na C10, these AUC_{0-1} values were 129.7 and 124.6 h $\cdot$ μ U/ml. Therefore, our results demonstrated that the optimum concentration of Na C10 was 1% as an insulin absorption promoter. Beyond the optimum concentration, the ACU_{0-1} value of insulin was decreased from 124.6 to 68.29 h $\cdot$ μ U/ml by insulin preparation with 5% Na C10.

We examined whether the peak time (t_{max}) of plasma IRI levels in the presence of Na C10 appeared within 15 min. Figure 4 shows a plasma IRI profile after an intranasal administration of 5 U/kg insulin with 1% Na C10 in rats.

Peak plasma IRI levels were reached 5 min after nasal administration of insulin with 1% Na C10 and the average IRI level was 778 μ U/ml. The IRI levels then decreased and reached 149 μ U/ml at 60 min after nasal admin-

TABLE II. *Effects of Fatty Acid Salts on Hemolytic Activity and Osmotic Pressure*

Solution	Conc. of fatty acid (%)	Hemolytic activity (%)	Osmotic pressure (mOsm)
Saline	0	0	270
Dist. water	0	100	9
Na caprylate	0.1	0.2	282
	0.5	1.6	330
	1.0	2.0	387
Na caprate	0.1	3.7	280
	0.5	73.7	322
	1.0	74.4	376
Na laurate	0.1	75.2	299
	0.5	68.2	313
	1.0	67.7	308

Each value is average of 3 experiments

TABLE III. *Gram Ions Sequestered by 1 mol of Fatty Acid Salts, Na Glycocholate or EDTA $\cdot$ 2Na*

Promoter	Ca ²⁺ (M)
	Promoter (M)
Na caprylate	0.021
Na caprate	0.351
Na laurate	0.416
Na glycocholate	0.135
EDTA $\cdot$ 2Na	1.078

Each value is average of 3 experiments

TABLE IV. *Residual Activity of Leucine Aminopeptidase in the Rat and Rabbit Nasal Mucosa in the Presence of Fatty Acid Salts or Glycocholate*

Promoter	Conc. of promoter (%)							
	0.1	0.5	1.0	5.0	0.1	0.5	1.0	5.0
	Rat				Rabbit			
Na caprylate	89 ±2.2	51 ±3.5	30 ±1.6	18 ±0.8	68 ±5.3	30 ±2.4	12 ±0.6	9 ±1.3
Na caprate	57 ±2.5	28 ±2.5	26 ±0.6	26 ±0.4	60 ±1.3	12 ±0.3	11 ±0.1	3 ±0.4
Na laurate	25 ±1.9	28 ±0.5	2 ±0.1		23 ±1.0	16 ±0.4	13 ±2.3	
Na glycocholate	37 ±0.9	25 ±2.7	21 ±0.3		31 ±1.6	14 ±0.3	14 ±0.8	

Each value is the mean ± S.E.M. for 3 experiments.

istration. The results indicated rapid absorption of insulin through the nasal mucosa in the presence of Na C10.

We investigated possible mechanisms of absorption promoting effect by fatty acid salts by examining hemolytic activity, osmotic pressure, chelating effect and inhibition of leucine aminopeptidase activity by fatty acid salt solutions. The effects of fatty acid salts on hemolytic activity and osmotic pressure are shown in Table II.

Hemolytic activity was higher with Na C10 and Na C12 than with Na C8. These activities are not necessarily dependent on fatty acid salt concentrations. Similar osmotic pressure values observed with these fatty acid salts do not correspond to the difference in the absorption promoting effect.

Since Ca ions are considered to constitute a tight junction structure of biomembranes, chelating ability of fatty acid salts for Ca ions was examined and is shown in Table III.

One M EDTA · 2Na was equivalent to 1 M Ca ions. In contrast, 1 M Na C8, Na C10 and Na C12 sequestered Ca ions of 0.02, 0.35 and 0.42 M, respectively. The results indicated that Ca ion binding ability was dependent on alkyl chain length of fatty acid salts.

The effects of absorption promoters on leucine aminopeptidase activity in rat and rabbit nasal mucosa are shown in Table IV.

The leucine aminopeptidase activity was decreased with increasing alkyl chain length of the fatty acid salts. The peptidase activity was inhibited effectively by the fatty acids at 0.5% or

above. These results suggest that fatty acid salts affect not only permeability of intercellular space by eliminating Ca ions of the nasal mucosa but also leucine aminopeptidase activity.

DISCUSSION

Non-parenteral administration of polypeptide hormones such as insulin or glucagon in place of parenteral administration is highly desired for therapy of insulin dependent diabetic patients or totally pancreatectomized patients. It is well known that absorption of polypeptides through nasal and rectal mucosa is significantly accelerated by adding absorption promoters.

In this study, we investigated nasal absorption promoting effects of medium chain fatty acid sodium salts which are known to be rectal absorption promoters of β -lactam antibiotics in rats.¹⁵⁾ We found that medium chain fatty acid sodium salts, such as Na C8, Na C10 and Na C12, significantly promoted absorption of insulin through nasal mucosa in rats. With Na C10, the peak plasma IRI level appeared at 5 min after nasal administration of insulin and the optimum concentration of Na C10 was 1% for nasal absorption of insulin. It was found that Na C10 was 1% for nasal absorption of insulin. It was found that Na C10 was the most effective absorption promoter among the three medium chain fatty acids. The nasal absorption of insulin in the presence of Na C10 occurred in a non-linear manner in contrast to Na glycocholate as shown in Table I. We cannot account for this, even if degradation of insulin was retarded due

to the inhibition of leucine aminopeptidase by Na C10. One possible reason can be in the calculation of AUC_{0-4} of insulin without plasma IRI level at 5 min. On the other hand, the t_{max} in the presence of Na glycocholate is expected to be about 15 min because t_{max} in the presence of Na deoxycholate, which is one of bile salts, was 15 min.²⁰⁾ Therefore, the absorption promoting effect by Na glycocholate resulted in a linear manner with insulin dose and in contrast, the effect by Na C10 might have resulted in a non-linear manner with insulin dose.

Insulin, in the presence of Na C10, was absorbed through rat nasal mucosa as rapidly as the rat rectal absorption of β -lactam antibiotics.¹⁵⁾ The optimum concentration (1%) of Na C10 in rat nasal absorption of insulin was less than that in rectal absorption of ampicillin containing Na C10 (2–5%).²¹⁾ These results indicate that these fatty acids can serve as nasal absorption promoters of insulin in the same way as rectal absorption promoters.

It is well known that the mucosal surface area of the nasal cavity is large and its vascular bed is responsible for the rapid passage of fluid and dissolved materials through the mucosa to the systemic circulation.²²⁾ Peptides can be absorbed from the nasal cavity in the same manner as from the rectal lumen. Therefore, it appears that the absorption promoting effect of medium chain fatty acid salts in the nasal mucosa is comparable to their rectal absorption promoting effect.

We investigated the absorption promoting mechanism of the fatty acid salts by examining possible correlation with hemolytic activity, osmotic pressure, chelating ability and inhibition of leucine aminopeptidase activity. The fatty acid salts showed higher hemolytic activity than glycocholate, but this result is not related to osmotic pressure.

Calcium and magnesium ions are essential to maintain a tight junction structure. Yata *et al.*¹⁹⁾ suggested that fatty acids have chelating ability for Ca ions and therefore serve to make the intercellular space more accessible by temporarily eliminating Ca ions from mucosal membranes. In the present study, we observed similar results in the chelating ability of the fatty acid salts for Ca ions.

In addition, insulin is subjected to degradation by protease such as leucine aminopeptidase

during its passage through the mucosal membrane.¹⁸⁾ The aminopeptidases show broad substrate specificity²³⁾ and wide tissue distribution.²⁴⁾ Aminopeptidase activity in nasal and rectal mucosa was of the same degree.²⁵⁾ In the present study, the leucine aminopeptidase activity in rat and rabbit nasal mucosa was inhibited more strongly by Na C10 and Na C12 than by Na C8 and the inhibition of the protease activity was not very different between fatty acid salts and sodium glycocholate. Our results suggest that the inhibitory effect of fatty acid salts on the proteolytic degradation of insulin in the nasal mucosa may be one possible mechanism of absorption promotion. Hemolytic activity, chelating ability and inhibition of leucine aminopeptidase activity of the three fatty acids did not correlate with the promoting effects of these fatty acids on insulin absorption. That is to say, Na C12 was stronger than Na C10 in these three activities but the promoting effect on insulin absorption was greater with Na C10 than with Na C12. In these studies, we adjusted the pH of the solution with buffers in experiments testing chelating ability and inhibition of leucine aminopeptidase activity except for hemolytic activity. Since Na C12 solution was more alkaline than Na C10, it may have caused a stronger effect on the red cell membrane at low concentrations of Na C12. On the other hand, the experimental solution used in the insulin absorption study was pH 7.4. Therefore, when the promoting effect on insulin absorption is compared with carbon chain length of fatty acids, Na C10 displayed the best promoting effect.

In conclusion, medium chain fatty acid salts with medium alkyl chain lengths (numbers of carbon 8 to 12) exerted the rapid nasal absorption promoting effect on insulin absorption and among them Na C10 showed the strongest promoting effect on insulin absorption. It was suggested that a possible mechanism was that fatty acids serve to make the intercellular space more accessible by temporarily eliminating Ca ions from the nasal mucosa and inhibit leucine aminopeptidase activity to decrease insulin degradation during passage through the nasal mucosal membrane. However, It seemed that high hemolytic activity of Na C10 was responsible for promoting action on the nasal mucosa. It is necessary, therefore to make further histological observations of acute and chronic damage of

nasal mucosa by optical and electron microscopes.

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