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Intratracheal delivery of insulin Absorption from solution and aerosol by rat lung

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Summary

In this study, we investigated the dynamics of insulin absorption from the lung with reference to bioavailability. Human insulin in 10 μl of aqueous solution and an insulin aerosol were administered into the exposed trachea of anesthetized rats. Blood samples were collected from the jugular vein at specified intervals and the plasma concentration of insulin was determined by an EIA. The relative bioavailability of insulin after intratracheal administration in 10 μl of pH 7.0 isotonic phosphate buffer and pH 3.0 isotonic citrate buffer was 13 and 42%, respectively, of that obtained after subcutaneous administration. Although insulin absorption in the presence of surfactants such as glycocholate, surfactin, and Span 85 was 3–4-times greater than that without surfactants, the co-administration of EDTA and salicylate, which previously enhanced the absorption of rectally administered insulin, did not increase the intratracheal absorption of insulin. The concomitant administration of nafamostat, a protease inhibitor, produced modest effects. The relative bioavailability of insulin given as an intratracheal aerosol was similar to that after subcutaneous administration. These observations indicate that the intratracheal route may be useful for the delivery of insulin.

Introduction

Many peptides and proteins of high molecular weight and low lipid solubility must be administered parenterally, with the exception of small peptides (Mol. Wt < 1000) and/or stable derivatives such as thyrotropin-releasing hormone, oxytocin, vasopressin, leuprolide, and enkephalin. However, the bioavailability of small peptides is only 10–40% after nasal administration and 0.3–1% after oral dosing (Moses 1964; Anderson and Arner, 1972; Mitsuma and Nogimori, 1984; Landgraf 1985; Pontiroli et al., 1985; Saito et al., 1985; Friedman and Amidon, 1991). Several reports indicate that insulin (Mol. Wt approx. 6000) is absorbed after rectal, oral, buccal, or nasal administration, however, its bioavailability is less than 10% by these routes. The addition of surfactants and/or proteolytic enzyme inhibitors to these dosage forms increases the bioavailability of insulin (Hirai et al., 1978, 1981; Ishida et al.,

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1981; Nishihata et al., 1981; Damgé et al., 1988). Unfortunately, there is considerable inter- and/or intra-individual variation in the bioavailability of insulin administered by the previously mentioned routes. Thus, a new delivery system that would be effective for administering insulin as well as other peptide and protein drugs is sought.

Wigly et al. (1971) and Elliott et al. (1987) delivered insulin to normal and diabetic patients by aerosol inhalation using a nebulizer. Unfortunately, the efficiency of absorption compared with that after subcutaneous injection was relatively low (10–25%). We previously reported the distribution and metabolism of several drugs in lung tissue from rats and rabbits following transpulmonary administration (Okumura et al., 1978, 1989; Hori et al., 1987; Yoshida et al., 1989, 1990). The relative bioavailability of insulin after aerosol inhalation in rabbits was approx. 40% (Yoshida et al., 1979). The present study was undertaken to describe the effect of surfactants, protease inhibitors, solution pH, and aerosolization on the absorption from rat lung of insulin as the model peptide.

Materials and Methods

Agents and preparation of aerosols

Recombinant human insulin (26.3 U/mg, Eli Lilly, Indianapolis, IN) was used. The insulin aerosol consisted of 5 mg insulin, 45 mg lactose, 2.4 g dichlorotetrafluoroethane (Daikin, Japan), 3.6 g dichlorodifluoromethane (Daikin, Japan) and 100 mg triolein sorbitan (Span 85, Nikko Chemical, Japan) (Yoshida et al., 1979). A metered dose aerosol valve (50 μl, Coster, Italy) was designed to release 62 μg of insulin per delivered dose. The mean particle size of insulin in the aerosol was 7.18 ± 4.46 μm (mean $\pm$ SD) for the volume distribution moment and 3.98 ± 2.19 μm for the length distribution moment using a particle analyzer (type 2600c, Malvern, England). Nafamostat mesylate (6-amidino-2-naphthyl *p*-guadininobenzoate dimethanesulfonate, Trii & Co., Japan), a protease inhibitor that inhibits trypsin, plasmin, and kallikrein, was used. Sodium glycocholate, surfactin, Na_2EDTA, carboxymethyl cellulose and sodium salicylate were supplied by Wako Pure Chemical (Osaka, Japan). Bacitracin was purchased from Sigma (St. Louis, MO) and gelatin from Upjohn Co. (Kalamazoo, MI). All other chemicals were of analytical reagent grade.

Animal experiments

Male Wistar rats weighing 250–300 g were used. They were anesthetized with pentobarbital (40 mg/kg, i.p.) during the experiments. Using a microsyringe with a thin needle (N-733, Hamilton, U.S.A.), 10 μl of isotonic phosphate buffer solution (pH 7.0) containing insulin (0.3 U/kg) was injected subcutaneously in a single dosage to each rat under the depilated skin of the back (Hori et al., 1983). The trachea was exposed and a microsyringe with silicon tubing (outer diameter 0.9 mm) was inserted through an incision made between the fifth and sixth tracheal rings caudal to the thyroid cartilage and to a depth of 12–15 mm according to the method of Enna and Schanker (1972). Recombinant human insulin at a dose of 3.0 or 6.0 U/kg in 10 μl of isotonic buffer of either pH 7.0 (phosphate buffer) with or without additives (surfactants or protease inhibitors) or pH 3.0 (citrate buffer) was injected through the silicon tubing into the trachea. The rats were maintained in a head-up position at an angle of 90° to horizontal for 30 s after the administration of insulin solution, and then at 15° during subsequent experiments. Plasma samples (100–150 μl) were collected from the jugular vein 0, 5, 15, 30, 60, 90, 120, 180, 240, and 300 min after insulin administration. Plasma levels of immunoreactive insulin (IRI) were estimated using an enzyme immunoassay (EIA) kit for human insulin (Abbott, North Chicago, IL). The cross-reactivity of this kit with rat insulin was less than 5%. Plasma glucose levels were measured by the *o*-toluidine method (Hyvärinen et al., 1962).

To estimate the dynamics of chlorofluorocarbon gases in the lung, an aerosol dye (Sudan blue B, 1-methylamino-4-*o*-tolylamino anthraquinone) was delivered as an aerosol into the trachea. The trachea was exposed and a stainless steel tube (outer diameter 1.0 mm) attached to a metered aerosol bottle was inserted through the site of the

tracheal incision to a depth of 10 or 20 mm. The lungs were removed 5 s after dosings and were homogenized in methanol. The homogenate was centrifuged at 10000 rpm for 5 min, and the concentration of dye in the supernatant was measured at 640 nm.

To estimate the dynamics of insulin in the lung, an insulin aerosol was delivered into the trachea. One dose of metered dose aerosol (MDI) was inhelated into the trachea. The trachea of the anesthetized rat was exposed, and a stainless-steel tube attached to a metered insulin aerosol bottle was inserted through the tracheal incision site to depths of 10 and 20 mm. The rats were maintained in the head-up position at an angle of 15° during these experiments. Plasma samples were collected from the jugular vein 0, 5, 15, 30, 60, 90, 120, 180, 240, and 300 min after insulin administration. Plasma levels of IRI were estimated using an EIA.

Bioavailability

The area under the plasma IRI concentration vs time curve (AUC) was calculated from 0 to 300 min using the trapezoidal method. The apparent relative bioavailability of insulin was calculated by comparing the AUC following transpulmonary administration under various conditions with that following subcutaneous administration.

Statistical analysis

Statistical analysis was performed using ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

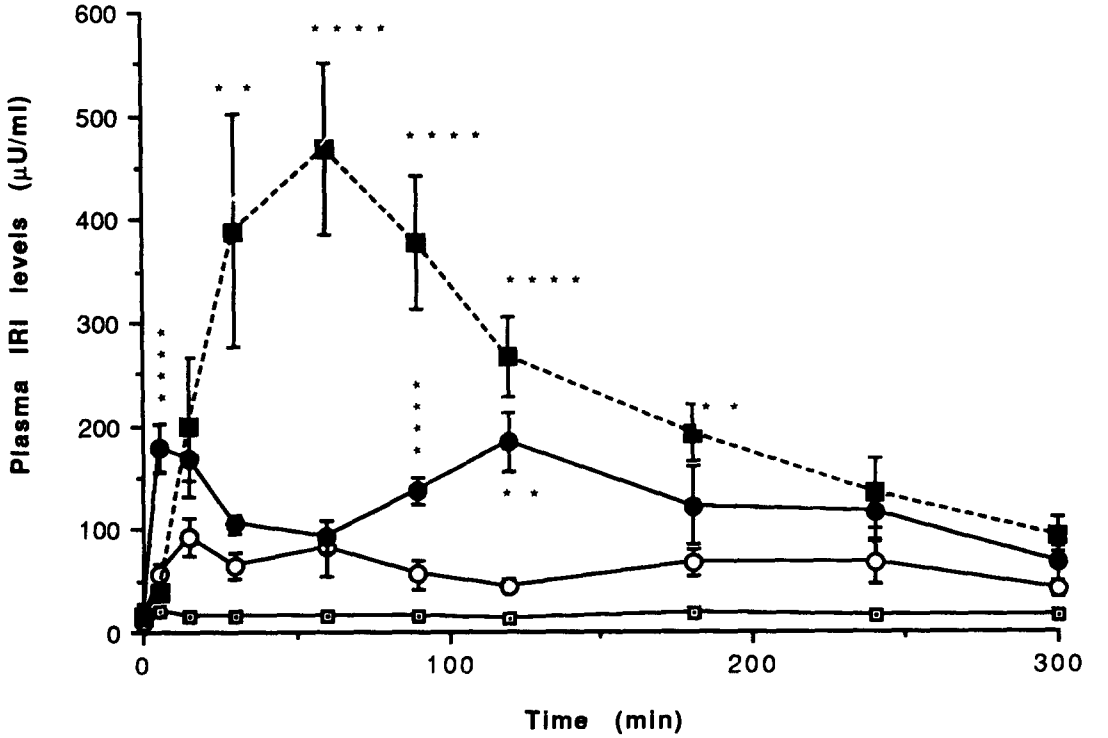

Fig. 1. Plasma IRI concentration-time profiles following the intratracheal administration of 10 μl insulin solution. Vertical bars indicate SE (⊡) Control (without insulin); (○) 3.0 U/kg insulin in pH 7 0 isotonic phosphate buffer; (●) 6.0 U/kg insulin in pH 7.0 phosphate buffer; (■) 3 0 U/kg insulin in pH 3.0 isotonic citrate buffer. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, and **** $p < 0.001$ compared with the intratracheal administration of 3 0 U/kg insulin in pH 7.0 isotonic phosphate buffer.

Results

Intratracheal absorption of insulin solution

After the intratracheal administration of recombinant human insulin (3.0 or 6.0 U/kg) in 10 μl of isotonic phosphate buffer pH 7.0 or in isotonic citrate buffer pH 3.0, the plasma IRI concentration was measured over 300 min. Fig. 1 shows the resulting plasma IRI concentration-time profiles. The two plasma IRI peaks appeared after the intratracheal administration of 3.0 or 6.0 U/kg in the pH 7.0 solution. The apparent relative bioavailability after these two doses was 13.1 and 14.1%, respectively, of that obtained after subcutaneous administration of 0.3 U/kg. The plasma IRI concentration after insulin was administered in the pH 3.0 solution was 4–5-times higher than that obtained with the pH 7.0 solution. The apparent relative bioavailability of insulin with the pH 3.0 solution was 41.6%.

Fig. 2 represents the effects of surfactants such as glycocholate, surfactin, and Span 85 on the intratracheal absorption of insulin. Plasma IRI levels increased significantly after the intratracheal administration of insulin in pH 7.0 phosphate buffer with 50 mM glycocholate or 10 mM surfactin. The addition of 1% Span 85 also promoted insulin absorption, while the addition of 1 mM surfactin had no effect. The bioavailability of insulin administered by the intratracheal route in a pH 7.0 solution containing 50 mM glycocholate, 10 mM glycocholate, 10 mM surfactin, 1 mM surfactin, or 1% Span 85 was 67.2, 31.4, 80.0, 15.1 and 40.6%, respectively (Table 1). The decrease in plasma glucose levels following the intratracheal administration of insulin with 50 mM glyco-

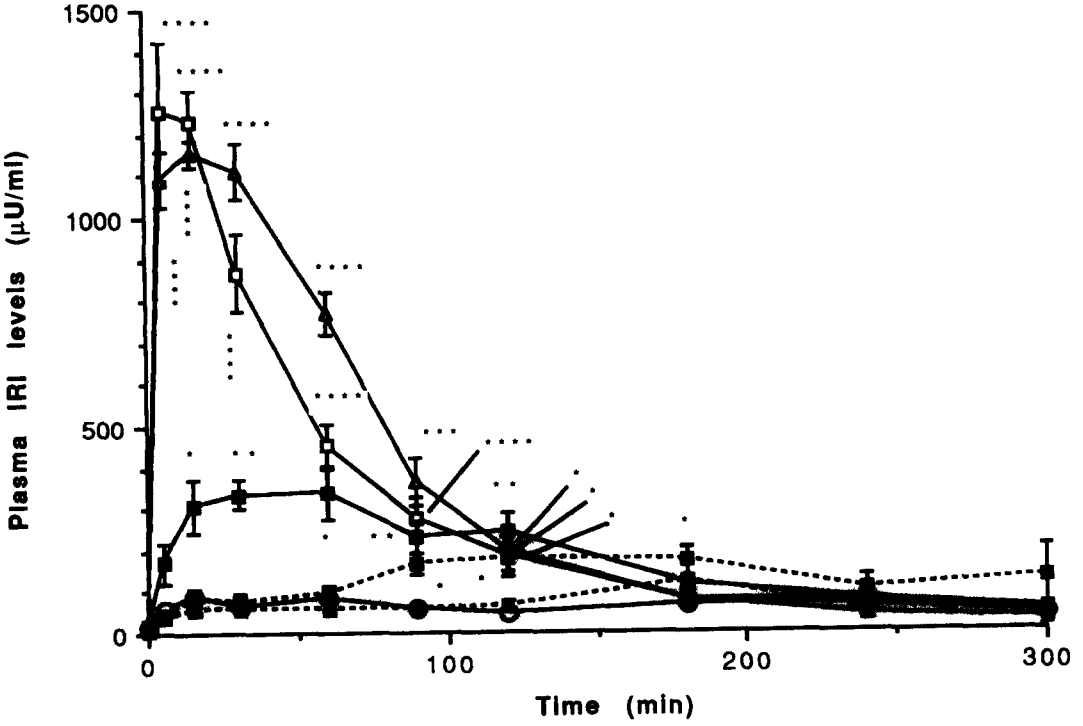

Fig. 2. Effects of glycocholate, surfactin and Span 85 on plasma IRI levels after the intratracheal administration of 3.0 U/kg insulin in pH 7.0 isotonic phosphate buffer. Vertical bars indicate SE. (○) 3.0 U/kg insulin in 10 μl isotonic phosphate buffer (pH 7.0); (■) insulin with 10 mM sodium glycocholate; (□) insulin with 50 mM sodium glycocholate; (▲) insulin with 1 mM surfactin; (△) insulin with 10 mM surfactin; (□) insulin with 1% Span 85. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.001$ compared with the intratracheal administration of 3.0 U/kg insulin in pH 7.0 isotonic phosphate buffer.

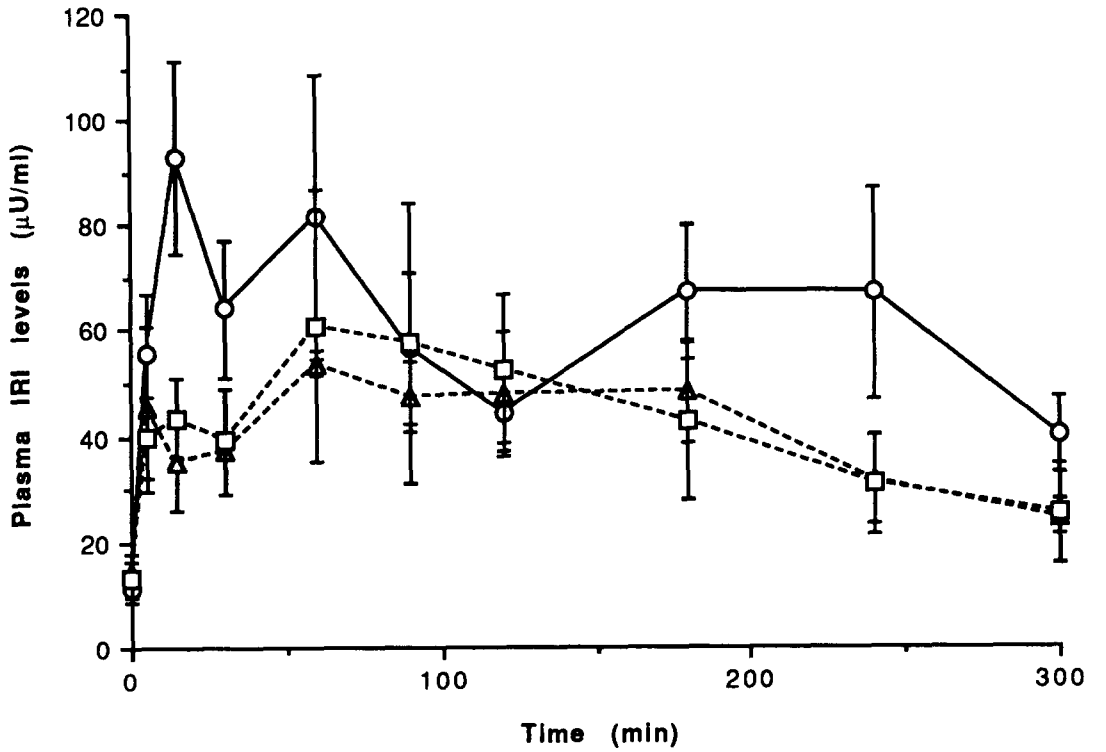

Fig. 3. Changes in plasma IRI concentration after the intratracheal administration of 3.0 U/kg insulin in pH 7.0 phosphate buffer with Na_2EDTA and sodium salicylate. Vertical bars indicate SE. (○) 3.0 U/kg insulin in 10 μl isotonic phosphate buffer (pH 7.0), (□) insulin 100 mM Na_2EDTA. (△) insulin with 100 mM sodium salicylate.

TABLE 1

Relative bioavailability of insulin after the intratracheal administration of solution or aerosol under various conditions

Condition	Number of experiments	AUC (min μU ml^{-1})		Relative bioavailability (%)
		Mean	±S.E.	
Insulin 0.3 U/kg (subcutaneous injection)	5	11643.2	1044.7	100.0
Insulin 3.0 U/kg in pH 7.0 solution (intratracheally)	5	15206.6	2733 5	13.1
+ Bacitracin, 1 mM	3	13548.0	2860.3	11.6
+ Nafamostat, 13 mM (intratracheally)	3	31883.3	9061.3	27.4
+ Nafamostat, 26 mM (intraperitoneally)	3	16287.3	2250.5	14.0
+ Carboxymethyl cellulose, 0.5%	6	16415.6	3367.1	14.1
+ Gelatin, 1%	3	6174.3	304.9	5.3
+ Glycocholate, 10 mM	8	36518.2	7603 7	31.4
+ Glycocholate, 50 mM	5	78227.8	11208.4 [b]	67.2
+ Surfactin, 1 mM	3	17628.5	3648.4	15 1
+ Surfactin, 10 mM	3	93128.5	8151 1 [b]	80.0
+ Span 85, 1%	3	47265 3	4833.0 [b]	40.6
+ EDTA, 100 mM	3	8913.6	3985 5	7.7
+ Salicylate, 100 mM	3	8129 7	1624.2	7.0
Insulin 6 U/kg in pH 7.0 solution	6	32725.3	5675.6	14.1
Insulin 3 U/kg in pH 3.0 solution	7	48460.0	9362.2 [a]	41.6
Aerosol of insulin 7.5 U/kg (depth = 20 mm)	5	284537.0	42488.0	97.8
Aerosol of insulin 7.5 U/kg (depth = 10 mm)	5	108656.0	18953.9	37.3
Insulin 3.0 U/kg in pH 7.0 solution + aerosol of Span	5	62783.8	26923.6	53.9

Statistical significance: [a] $p < 0.01$ and [b] $p < 0.001$ compared with insulin 3.0 U/kg in pH 7.0 solution (intratracheally).

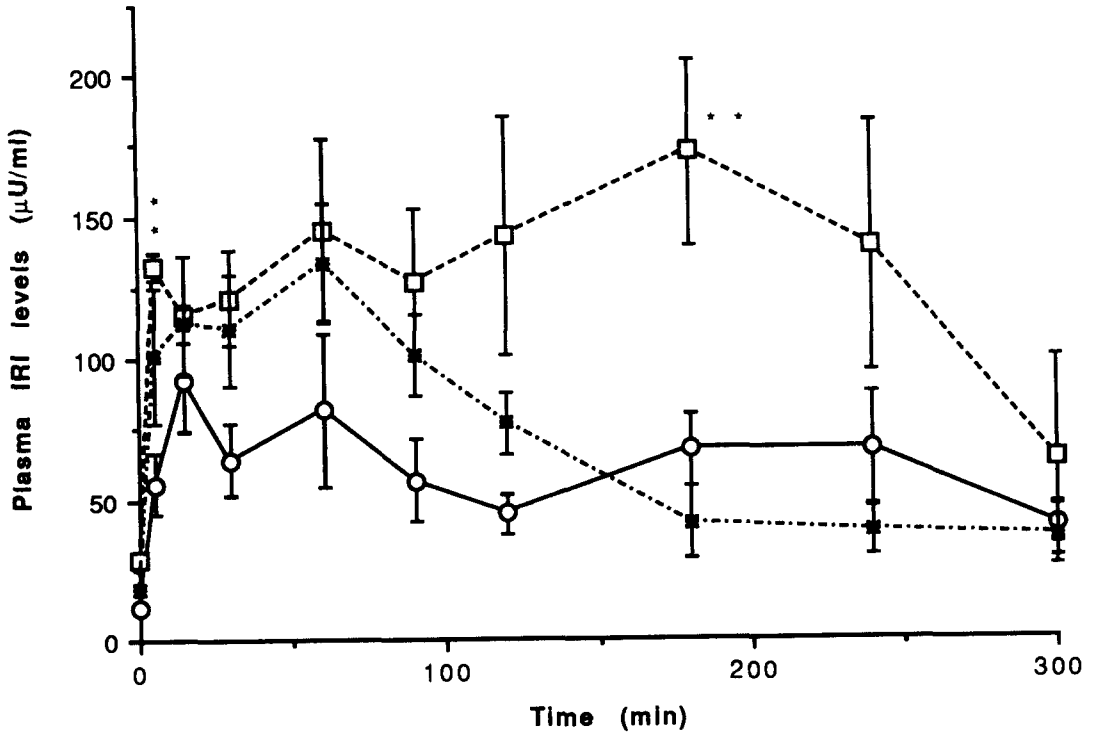

Fig. 4. Effect of nafamostat on plasma IRI concentration after the intratracheal administration of 3.0 U/kg insulin in pH 7.0 phosphate buffer. Vertical bars indicate SE. (○) 3.0 U/kg insulin in 10 μl isotonic phosphate buffer (pH 7.0); (□) intratracheal administration of insulin with 13 mM nafamostat; (×) intratracheal administration of insulin with simultaneous intraperitoneal administration of 26 mM nafamostat. Statistical significance: ** $p < 0.01$ compared with the intratracheal administration of 3.0 U/kg insulin in pH 7.0 isotonic phosphate buffer

cholate and 10 mM surfactin exceeded that following the administration of the control insulin solution (data not shown). Salicylate and EDTA, which enhance the absorption of insulin from the rectum, did not increase intratracheal absorption (Fig. 3).

The apparent relative bioavailability of insulin following intratracheal administration with the serine protease inhibitor nafamostat (13.0 mM) in pH 7.0 isotonic phosphate buffer was approximately twice that obtained with only the insulin solution. However, the apparent relative bioavailability of intratracheal insulin was not increased by the intraperitoneal administration of nafamostat (26.0 mM) in pH 7.0 isotonic phosphate buffer (Fig. 4 and Table 1). The addition of bacitracin (1.0 mM) had no effect on bioavailability.

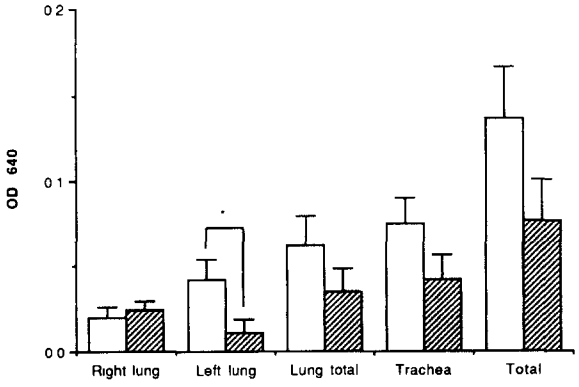

Fig. 5. Distribution of dye in the rat lung after the intratracheal administration of Sudan blue B aerosol. Vertical bars indicate SE ($n = 3$) Open columns indicate aerosol dye through 20 mm tubing in rat trachea, hatched columns indicate aerosol dye through the tube at depth of 10 mm from the site of the tracheal incision Statistical significance: * $p < 0.01$

The addition of 1% gelatin to the insulin solution decreased bioavailability from the lung (Table 1). Thus, increases in viscosity were associated with decreases in the amount of insulin absorbed from the lung.

Pulmonary absorption of insulin delivered by intratracheal aerosol

To evaluate the dynamics of chlorofluorocarbon gas in the lung, an aerosol dye was delivered through the tracheal incision to a depth of 10 or 20 mm. The amount of dye distributed in the left lung at a depth of 20 mm was 3-times greater than that at 10 mm. The amounts of dye distributed in the trachea and total lung at depths of 20 mm were twice those at depths of 10 mm (Fig. 5). However, the amount of dye distributed in the right lung did not differ between the two depths following administration into the trachea. These observations show that the depth of placement of the aerosol tube in the trachea affects the amount and nature of drug distribution.

Span 85, which was added to the insulin aerosol to stabilize the insulin particles in the chlorofluorocarbon gas, enhanced the absorption of insulin 3.0 U/kg in a pH 7.0 solution (Fig. 2). Accordingly, the effect of an aerosol with only Span 85 (no insulin) was studied. After the simultaneous intratracheal administration of Span 85 aerosol and 3.0 U/kg of insulin solution, the plasma level of insulin increased and the relative bioavailability of insulin was approx. 4-times higher than that observed with only the insulin solution (Table 1). Thus, Span 85 increased the absorption of insulin via the intratracheal route.

The insulin aerosol was delivered through the tube at depths of 10 and 20 mm from the site of the tracheal incision (Fig. 6). The plasma IRI

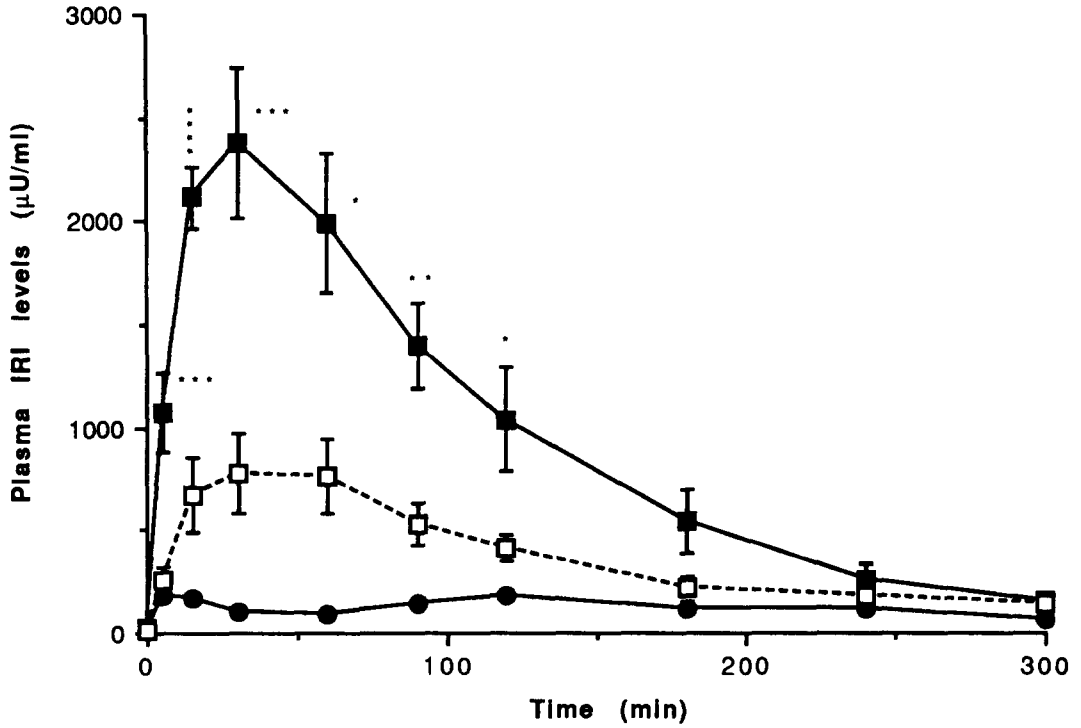

Fig. 6. Plasma IRI concentration profiles after the intratracheal administration of insulin aerosol. Vertical bars indicate SE (●) 6.0 U/kg insulin in 10 μl isotonic phosphate buffer (pH 7.0), (■) 7.5 U/kg insulin aerosol through 20 mm tubing in rat trachea; (□) 7.5 U/kg insulin aerosol through 10 mm tubing in rat trachea. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.001$ compared with the intratracheal administration of insulin aerosol through the tube at depths of 10 and 20 mm from the site of the tracheal incision.

levels increased rapidly following delivery of insulin aerosol at both depths. The bioavailability of insulin from aerosol delivered at depths of 10 and 20 mm into the trachea was 37.3 and 97.8%, respectively.

Discussion

When insulin was administered intratracheally in doses of 3.0 or 6.0 U/kg in the pH 7.0 solution containing no absorption enhancers, this apparent relative bioavailability was greater than values previously determined via the oral, buccal, rectal and nasal routes (Hirai et al., 1978, 1981; Ishida et al., 1981; Nishihata et al., 1981; Damgé et al., 1988). These observations indicate that transpulmonary administration of insulin without enhancers may have considerable potential for systemic delivery as compared with rectal and nasal insulin administration. In this study, two plasma IRI peaks appeared after the intratracheal administration of insulin (Fig. 1). Since insulin occurs in both a high and a low molecular weight form (hexamer and monomer, respectively) at neutral pH (Brange 1987), the first plasma IRI peak may indicate the absorption of the monomer form and the second may represent that of the hexamer form.

The plasma IRI concentration-time profile following the intratracheal administration of insulin (3.0 U/kg) in the pH 3.0 solution showed rapid increases, and the apparent relative bioavailabilities were 3-times greater than those in the pH 7.0 solution (Fig. 1 and Table 1). The insulin molecule exists only in the monomer form in acidic solutions. Therefore, this finding suggests that the pulmonary absorption of the monomeric form of insulin was very rapid, and/or that the lung tissue was damaged by the acid solution. A similar result was previously reported after nasal insulin absorption (Hirai et al., 1978).

Surfactants such as glycocholate, surfactin and Span 85 enhanced the absorption of insulin from the lung in a dose-dependent fashion (Fig. 2). Several reports indicate that surfactants can increase the absorption of nasally, buccally and rectally administered insulin (Ichikawa et al., 1980; Hirai et al., 1981; Moses et al., 1983; Gorden et al., 1985; Aungst and Rogers 1988). Our present results may indicate lung tissue barrier and/or the endogenous pulmonary surfactant damage by exogenous surfactants.

EDTA and salicylate, which were previously shown to enhance the absorption of rectally but not nasally or buccally administered insulin (Aungst and Rogers 1988), did not enhance intratracheal insulin absorption in the present study (Fig. 3). EDTA and salicylate increase paracellular transport by affecting the permeability of tight junctions as a consequence of the removal of calcium (Cassidy and Tidball et al., 1967; Suzuka et al., 1987). The present results confirmed the lack of susceptibility of lung tissue membrane to the promotion of paracellular transport.

The relative bioavailability of insulin after intratracheal administration with nafamostat, a serine protease inhibitor, was approximately twice that without nafamostat. However, the relative bioavailability of intratracheal insulin after simultaneous intraperitoneal administration of nafamostat was not increased. Furthermore, bacitracin did not affect the bioavailability of insulin after intratracheal administration (Fig. 4 and Table 1). On the other hand, in our previous reports (Komada et al., 1985; Takeyama et al., 1991), both nafamostat and bacitracin inhibited the degradation of insulin given by subcutaneous injection. These findings demonstrate that the proteolytic enzyme activity of the lung in degrading insulin is weaker than that of rectal (Hayakawa et al., 1987) and subcutaneous tissues (Hori et al., 1983; Okumura et al., 1985).

The distribution profile of aerosolized dye following intratracheal administration directly indicates the distribution of chlorofluorocarbon in the lung (Fig. 5). The distribution of dye in the right lung did not differ following administration at either depth into the trachea. However, the amount of dye distributed in the left lung, which was delivered via a tube placed in the trachea to a depth of 20 mm, was approx. 3-times greater than that delivered at the 10 mm depth. The tracheobronchial tree is an asymmetrical airway (Chang and Menon 1985), although the trachea leading to the right lung is straighter and has a

greater diameter than the trachea leading to the left lung. In addition, the right lung of the rat consists of small separate lobules. Therefore, the depth of aerosol delivery affected the amount of dye distributed in the left lung but may not have contributed to the amount distributed in the right lung. The amount of dye in the trachea differed under the various experimental conditions. With delivery through the shallow tube insertion, the aerosol escaped through the tracheal incision. Thus, the depth of aerosol inhalation contributed to the absorption of insulin.

Span 85 is required to stabilize insulin in the aerosol form (Morén 1985). This agent is included in many aerosols such as the bronchodilator aerosols that are now widely used. Span 85 enhanced the pulmonary absorption of insulin from aqueous solution (Fig. 2); thus, the effect of Span 85 aerosol without insulin was studied. After the intratracheal administration of Span 85 aerosol followed by insulin solution, plasma IRI levels rapidly increased (Fig. 6) and the relative bioavailability of insulin increased approx. 4-times above that seen with the insulin solution alone (Table 1). Thus, Span 85 is not only required to stabilize insulin in the aerosol form, but it is also effective in enhancing insulin absorption after intratracheal administration of the aerosol. Niven and Byron (1990) previously reported that Span 85 also enhanced the absorption of fluorescein from the airways when dosed from the metered dose inhaler containing 5% (w/w) Span 85.

The size of the particles contained in the aerosol influences their distribution in the lung; particles that are 2–5 μm in diameter constitute the greatest sediment in the alveoli (Brain 1985; Gonda 1981). In the present study, the mean size of the insulin particles was approx. 4 μm.

A metered dose aerosol inhaler (50 μl) designed for clinical use was employed in this preliminary insulin aerosol inhalation study. The volume of aerosol was suitable for human lung inhalation, but was too great for rat lung inhalation. However, no macroscopic lung injury was observed after the experiment. Plasma levels of IRI increased rapidly after aerosol delivery via the tubes placed 10 and 20 mm into the trachea. The bioavailability of insulin under these conditions was 37.3 and 97.8%, respectively. The apparent relative bioavailability of insulin increased approx. 2-fold after the administration of aerosol which was delivered via a tube placed in the trachea to a depth of 20 mm. This finding agrees with the observation in the dye distribution experiment. The relative bioavailability of insulin given by an intratracheal aerosol administration was similar to that of insulin given by subcutaneous injection, since insulin particles may be delivered by the pressurized chlorofluorocarbon gas flow into the alveoli.

We conclude that: (1) the relative bioavailability of insulin after intratracheal administration in 10 μl of pH 7.0 isotonic phosphate buffer and pH 3.0 isotonic citrate buffer was 13 and 42%, respectively, of that achieved with subcutaneous administered insulin; (2) insulin absorption was enhanced by surfactants such as glycocholate, surfactin and Span 85 to 3–4-times the level absorbed without surfactants; (3) EDTA and salicylate, which previously enhanced the absorption of rectally administered insulin, did not increase the intratracheal absorption of insulin; (4) the effects of a concomitantly administered protease inhibitor were modest; and (5) the relative bioavailability of insulin given as an intratracheal aerosol was similar to that of insulin given subcutaneously.

The results of this study suggest that the intratracheal administration of insulin is an effective route for systemic delivery that merits further evaluation.

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