Insulin Across Respiratory Mucosae by Aerosol Delivery

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SUMMARY

Insulin, a protein of about 5,700 molecular weight, was delivered by aerosol inhalation to three normal volunteers and to four patients with diabetes mellitus. Direct evidence of absorption of insulin across mucosae of the respiratory tract was an increase in plasma IRI. Biologic activity of insulin absorbed by inhalation was shown by hypoglycemia temporally correlated with the increase in plasma IRI. No untoward reactions were observed. DIABETES *20*:552-56, August, 1971.

Many inhaled substances have been shown to cross respiratory mucosae and to act systemically. Common examples include nicotine and marijuana inhaled from cigarette smoke.[1] Adrenal cortical steroids, when applied intranasally, are absorbed and cause adrenal suppression.[2] Vasopressin may be administered by nasal spray in the treatment of diabetes insipidus.[3] Several nonreplicating antigens, such as inactivated influenza[4] and parainfluenza[5] viruses, and tetanus toxoid,[6] have been shown to stimulate systemic antibody formation following aerosolization into the respiratory tract.

The purpose of this study was to investigate whether insulin, delivered to humans by aerosol, crossed respiratory mucosae and retained biologic activity.

METHODS

Pre-human studies in rabbits

Initially, effects of insulin given by aerosol were determined in rabbits. One group of rabbits received insulin by placing the animal's head in a plastic bag into which 400 units of insulin were aerosolized using a Devilbiss No. 40 nebulizer (The Devilbiss Company, Toledo, Ohio). Control rabbits received no insulin throughout the experiment. Other rabbits received 20 units of insulin intravenously to test biologic activity of the insulin used. Blood glucose[7] on samples from ear veins was monitored approximately every fifteen minutes for two hours. Regular insulin (80 U./ml.) was used in all rabbits.

Human experimentation

In studies with humans, each subject was admitted to the Clinical Research Center, Shands Teaching Hospital, University of Florida College of Medicine, and informed consent was obtained. For safety, a saline infusion was maintained throughout the experiment, and solutions of 50 per cent glucose, adrenalin, and glucagon were available. Each subject was supine throughout the experiment and had fasted from midnight. A standard oral glucose tolerance test[8] was performed on each subject, and a tolbutamide stimulation test[9] was done on each subject in the diabetic group.

During the aerosol delivery of insulin, the subject inhaled a nebulized mist of Regular pork-beef insulin which contained 500 U./ml. (Eli Lilly Co., Indianapolis, Ind.). Different means of producing small particle size were studied. It was found that the ultrasonic nebulizer destroyed the biologic activity of the insulin molecule. Therefore, a Devilbiss No. 40 nebulizer, which produces a mean particle size of approximately 2 μ, was used. Blood samples were taken at regular intervals, beginning with three control samples before aerosolization, on which plasma immunoreactive insulin (IRI),[10] glucose,[11] and, in one patient, free fatty acids[12] were determined.

In an attempt to quantitate the delivery of insulin by aerosolization, changes in plasma IRI and glucose were compared with those following the intravenous administration of insulin (0.1 and 0.2 U./kg.) in the four patients with diabetes.

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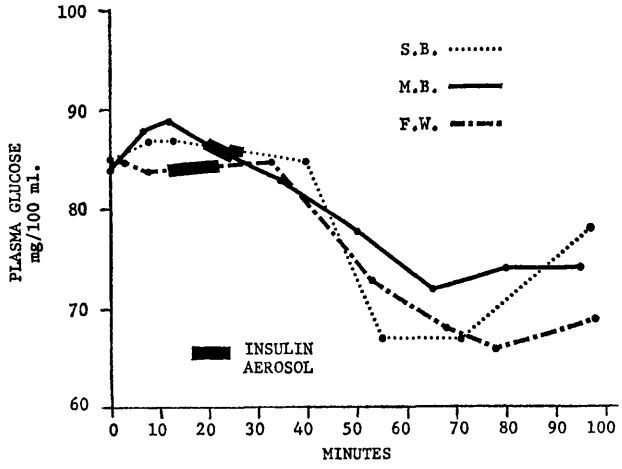

FIG. 1. Normal Subjects: Change in plasma glucose following insulin by aerosol.

RESULTS

Studies in rabbits

In control rabbits which received no insulin, blood glucose concentration remained stable throughout the experiment. Rabbits which received insulin by aerosol showed a decrease in blood glucose from approximately 150 to 90 mg./100 ml. over a two-hour period following aerosolization. Biologic potency of the insulin used was shown by the observation that rabbits which received 20 units of insulin intravenously became hypoglycemic (blood glucose less than 45 mg./100 ml.), had convulsions, and died within one hour.

Studies in normal man

Studies in man were done first in normal, healthy male medical students between ages twenty-three and twenty-seven. Each had a normal glucose tolerance test. The hypoglycemic response suggested that biologic activity of insulin was demonstrable after aerosolization of insulin (figure 1). No untoward reactions occurred.

Studies in humans with diabetes mellitus

Four patients with diabetes mellitus were selected. Clinical and laboratory features are shown in table 1. None had a history of allergy or symptomatic pulmonary disease. Each subject had a diabetic glucose tolerance response, and each was without ketonuria. Fasting plasma glucose ranged between 140 and 320, and plasma IRI between 10 and 26 μU./ml. (table 1). There was minimal or no increase in plasma IRI following tolbutamide stimulation.

After insulin by aerosol, there was an increase in plasma IRI and a decrease in plasma glucose concentration when compared with saline control periods. Variations in response are best seen in figures 2-6, which show the observed changes in detail.

The results in patient L. G. (figure 2), a diabetic of recent onset who had not received previous treatment with insulin or oral hypoglycemic agents, illustrate the prompt increase in IRI in peripheral blood; the concomitant hypoglycemia indicates its biologic effectiveness. Fifteen minutes after the five-minute inhalation, plasma IRI had increased from 14 to 77 μU./ml. By this time, plasma glucose had decreased from 142 to 84 mg./100 ml.; peak hypoglycemia of 38 mg./100 ml. was observed at thirty minutes (figure 2).

The absolute changes in plasma IRI and glucose were less in patients C. S. and C. T. With the latter patient, cooperation and effective inhalation were more variable.

TABLE 1

Insulin by aerosol to humans with diabetes mellitus
Selected clinical and laboratory features in patients with diabetes mellitus.
The post-aerosol plasma IRI and glucose are peak values after insulin by aerosol.

Subject	Age	Sex	Ht.	Wt. (kg.)	Duration Diabetes (yr.)	Previous Treatment: Diet	Sulfonylurea	DBI	Insulin	Dose Insulin Aerosol (units/5 min.)	Plasma IRI Pre-Aerosol (μU./ml.)	Plasma IRI Post-Aerosol (μU./ml.)	Plasma Glucose Pre-Aerosol (mg./100 ml.)	Plasma Glucose Post-Aerosol (mg./100 ml.)
1. L. G. (fig. 2)	30	M	5′10″	76.8	<1/12	+	0	0	0	250	14	77	140	38
2. C. T. (fig. 3)	64	M	6′1″	86.4	2	+	+	+	0	250	21	38	257	198
3. C. S. (fig. 4)	58	F	5′5″	68.2	10	+	+	+	0	250	26	46	320	250
4. N. T. (fig. 5)	64	M	5′10″	76.4	26	+	+	0	+	250	10	48	257	209

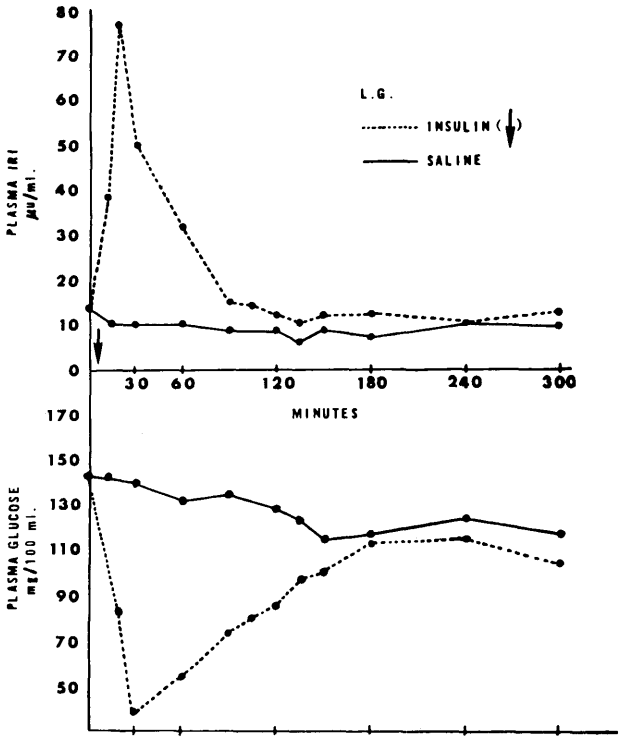

FIG. 2. Diabetes—Patient L. G.: Plasma IRI and glucose after aerosol delivery of insulin or saline.

Patient N. T. had received insulin for a short period fifteen years previously; plasma antibodies to insulin were not detectable. The fourfold increase in plasma IRI indicated absorption of insulin. Also, in N. T., a decrease in plasma FFA from 1.0 to 0.77 mM by 60 minutes and to 0.58 mM at 120 minutes after insulin aerosol was additional evidence of biologic effectiveness. Temporal changes in plasma glucose and IRI are shown in figure 3.

Quantitation of aerosol method

To estimate the efficiency of the aerosol method, an attempt was made to determine what per cent of the total dosage delivered by aerosol crossed the respiratory mucosae. Regular insulin was given intravenously to the four patients with diabetes; their hypoglycemic response to this known quantity of insulin was compared to that after insulin aerosol. Each received 0.1 and 0.2 U./kg. body weight.

Table 2 shows plasma insulin as reflected by the area under plasma IRI curves. With aerosol delivery of 250 units, or about 3 units/kg., the plasma IRI area was 7-16 per cent of that after intravenous injection of 0.2 U./kg. Roughly, this would indicate absorption of about

TABLE 2

Quantitation of aerosol method
Comparison of plasma IRI curves following delivery of insulin by aerosol inhalation and intravenously.

Subject	Plasma IRI* N. T.	L. G.	C. S.	C. T.
Amount Insulin Administered				
0.2 U./kg. I.V.	0.11*	0.16	0.14	0.14
0.1 U./kg I.V.	0.05	0.09	0.05	0.12
Approx. 3 U./kg aerosol	0.01	0.025	0.01	0.01

*Refers to area of plasma IRI curves measured by weight of paper.

0.01-0.03 U./kg. from the aerosol dose. A comparative estimate of the biologic activity of the insulin which crossed the respiratory mucosae after aerosolization is shown in figure 6. In L. G., the hypoglycemia after aerosol delivery of insulin was comparable to that after 0.2 U./kg. intravenously. With N. T., the peak hypoglycemia observed approached that after 0.1 U./kg., intravenously, but was more delayed.

DISCUSSION

The prompt increase in plasma IRI following the delivery of insulin by aerosol inhalation to patients with

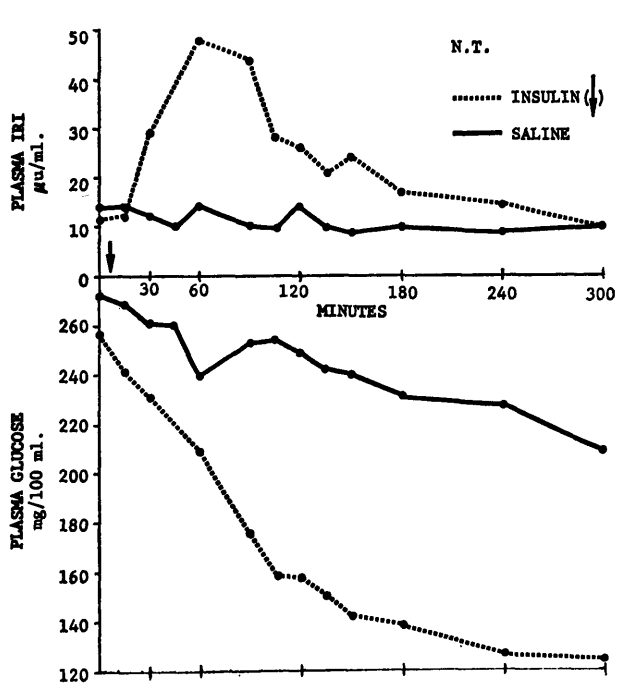

FIG. 3. Diabetes—Patient N. T.: Plasma IRI and glucose after aerosol delivery of insulin or saline.

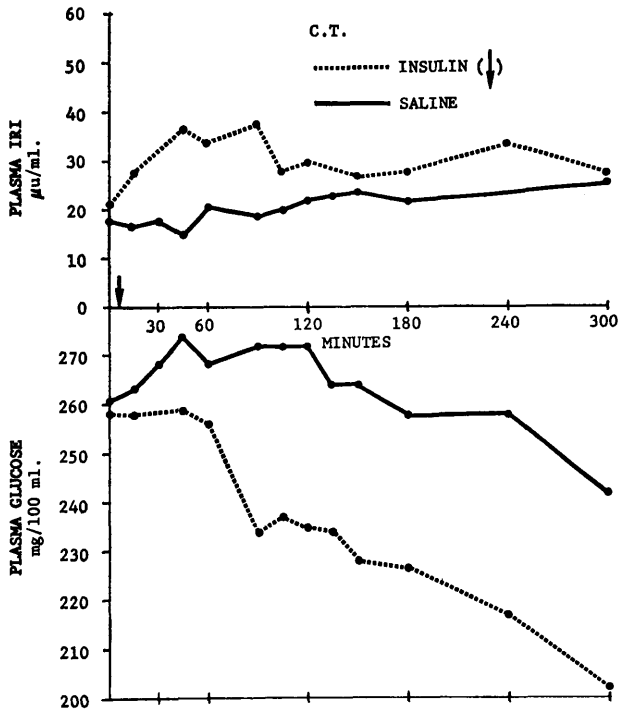

FIG. 4. Diabetes—Patient C. T.: Plasma IRI and glucose after aerosol delivery of insulin or saline.

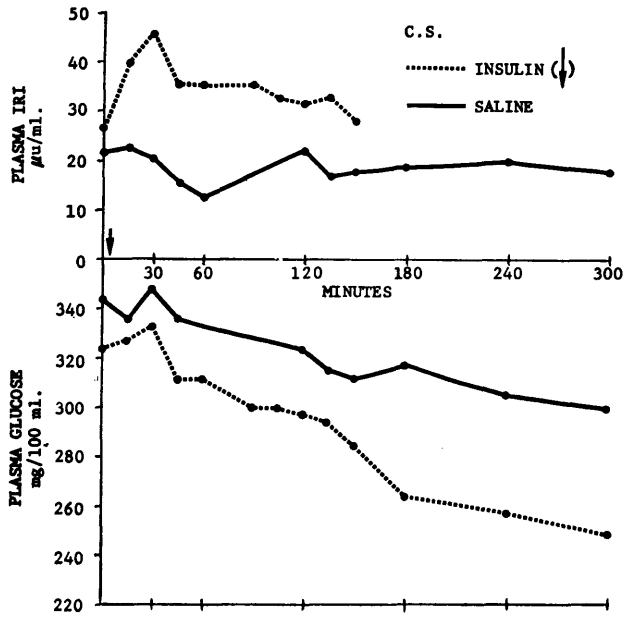

FIG. 5. Diabetes—Patient C. S.: Plasma IRI and glucose after aerosol delivery of insulin or saline.

diabetes mellitus showed that insulin crossed the mucosae of the respiratory tract. The observed hypoglycemia indicated that insulin retained biologic activity. This was further supported by the decrease in FFA in one patient (N. T.). In each patient, results after insulin aerosolization were compared with a control period following saline by aerosol.

The absorption of insulin was rapid as shown by a detectable increase in plasma IRI within fifteen minutes (patient C. S., figure 5) and a peak increment

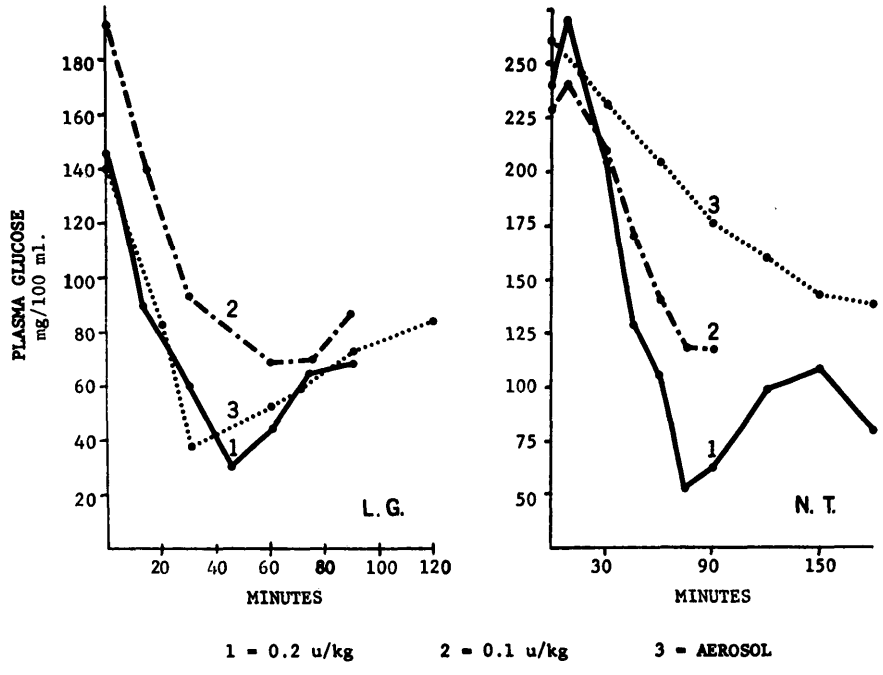

FIG. 6.
Diabetes: Plasma glucose change after insulin by aerosol and intravenously (0.1 and 0.2 U./kg.). After aerosol delivery, note the rapid decrease in patient L. G., and a slower decline in patient N. T.

within thirty minutes (patients L. G., figure 2, and C. S., figure 5). Peak responses were more delayed in patients C. T. (figure 4) and N. T. (figure 3).

Hypoglycemia was temporally correlated with the increase in plasma IRI. Patient L. G. (figure 2) provides a good illustration. In this patient the peak rise in plasma IRI and maximal hypoglycemia were observed within thirty minutes. Further, the amount of insulin absorbed by the respiratory route produced as much hypoglycemia, and at a similar rate, as was observed with 0.1 or 0.2 U./kg. (figure 6). Similar comparisons in the other three patients showed that a lesser amount of insulin was absorbed (table 2), and hypoglycemia was more delayed, and less, than that seen after the intravenous administration of insulin.

Quantitative estimates suggested that the delivery of insulin by the aerosol route was of low efficiency. Approximately 10 per cent of the aerosolized insulin was recovered, based on comparisons of plasma IRI (table 2). Additional studies will be required to delineate factors responsible for the low and variable efficiency. For example, it will be necessary to determine how much insulin is lost by sticking to the glass nebulizer system, and how much is lost by exhalation. Better training of patients could result in enhanced delivery to absorptive sites.

It was not the intent of this study to propose this route of insulin administration for the patient with insulin-dependent diabetes. Additional studies to improve the efficiency and dependability of delivery, and confirmation of safety with long-term use would be required prior to consideration for clinical use. No adverse effects were observed in the acute studies reported, except for symptomatic hypoglycemia.

ACKNOWLEDGMENT

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