# Nasal Absorption of Insulin in Dogs

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SUMMARY

**The intranasal application of an insulin solution in dogs resulted in the rise of plasma immunoreactive insulin and in dose-dependent hypoglycemia. The absorption of insulin from this site was found to be enhanced when insulin was dissolved in an acid medium. In addition, when an insulin preparation with some surfactant was used, the effectiveness of nasally administered insulin was 25 to 30 per cent of that achieved with intravenously administered insulin.** DIABETES *27*:296-99, March, 1978.

It is generally accepted that, for therapeutic use, a substance such as peptide cannot be given orally to patients as it will be destroyed by the digestive juices. Insulin, for example, can be given effectively only by injection because it is degraded by proteolytic digestion in the gastrointestinal tract when taken orally. This is a problem for diabetic patients who are unable to inject insulin for themselves or are in need of long-term insulin therapy.

Numerous efforts have been made to find an effective route for the administration of insulin other than parenteral injection, such as the oral,[1-3] buccal,[4] and tracheal routes.[5] However, the polypeptide nature of insulin has limited the development of nonparenteral application as a practical route of administration.

Absorption of small peptides through the nasal mucosa has been used in the treatment of diabetes insipidus.[6] LH-releasing hormone has also been shown to stimulate LH release when administered as nasal drops in man.[7]

The purpose of this study was to investigate whether insulin could be absorbed through the nasal mucosa and display its biologic activity in dogs before studying this method in human subjects.

## METHODS

### *Materials and Insulin Preparations*

Crystalline pork insulin (25.5 U./mg., Shimizu Seiyaku Co., Ltd., Japan) was dissolved or suspended in 0.01 M acetate buffer (below pH 6.0) and in 0.01 M phosphate buffer (above pH 6.0) for the intranasal application at a concentration ranging from 2 U./0.1 ml. to 50 U./0.1 ml. In order to facilitate the nasal absorption of insulin, surfactants such as saponin obtained from tea leaves (Wako Pure Chem. Ind., Ltd., Japan), sodium glycocholate (Yoneyama Chem. Ind., Ltd., Japan) or polyoxyethylene-9-lauryl ether (BL-9, Nikko Chem. Ind., Ltd., Japan) was added to insulin solutions at a concentration of 1 per cent. For the intravenous, intramuscular, and subcutaneous applications, regular insulin (40 U./ml., Shimizu Seiyaku Co., Ltd., Japan) was diluted with physiologic saline in a concentration ranging from 0.5 U./ml. to 5 U./ml.

### *Administration of Insulin Preparation in Dogs*

Four or five male beagle dogs weighing 8 to 10 kg., fasted previously for 24 hours, were anesthetized with the intravenous injection of 30 mg./kg. of sodium pentobarbital forty minutes before the experiments. Then 0.1 ml. of the insulin preparation was administered to the nasal cavity in the form of a nebulizer spray.

Blood in a volume of 0.4 ml. was sampled in a heparinized syringe from the foreleg veins at 30 minutes before and 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 hours after the administration of insulin and the plasma was separated by centrifugation at 5° C. The venous blood was also sampled similarly when insulin was injected intravenously, intramuscularly, or subcutaneously.

### *Estimation of Plasma Immunoreactive Insulin and Plasma Glucose*

Change in plasma glucose level was mainly estimated by the method with o-toluidine boric acid[8] to measure the nasal absorption of insulin. Plasma immunoreactive insulin was also determined by use of Radioimmunoassay Kit (Radiochemical Center, Amersham, England) devised by Randle and Hales,[9] to prove that insulin can be absorbed from the nasal mucosa into systemic blood stream as the unchanged form.

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## RESULTS

### *Absorption of Insulin from the Nasal Mucosa*

As shown in figure 1, in dogs that received insulin nasally at a dose of 50 U./dog (pH 3.1), the plasma immunoreactive insulin (IRI) increased from 32.8 to 296.7 μU./ml. at 30 minutes and plasma glucose level decreased from 90.8 to 20.4 mg./100 ml. (100 to 24.7 per cent) at two hours after the administration, and these were followed gradually to the initial levels with the passage of time. In control dogs, however, plasma glucose level was constant throughout the experimental period.

### *Effect of pH of the Preparation on the Nasal Absorption of Insulin*

Both the extent and duration of hypoglycemia induced by the intranasal application of insulin depended on the pH of the preparation. Absorption of insulin from the nasal mucosa was enhanced significantly when it was dissolved in an acid medium rather than in a neutral one. Plasma glucose level after the intranasal application of the insulin preparation with pH 3.1 or 3.7 at a dose of 50 U./dog was about 25 per cent of the initial level at one or two hours after the administration and recovered gradually but lasted for more than six hours. In figure 2, the relationship between pH and the decrement of the plasma glucose level (total decrease) from zero to six hours is shown. At pH 6.1, close to the isoelectric point of insulin, no or only slight hypoglycemia appeared. But at pH 3.1, the decrement of the plasma glucose level (total decrease) corresponded to about 55 per cent.

### *Effect of Surfactant on the Nasal Absorption of Insulin*

When the nasally applied 50 U./dog of the insulin preparation with pH 7.4 was combined with either saponin, sodium glycocholate, or BL-9 at a concentration of 1 per cent, the resultant hypoglycemia was enhanced significantly in either extent or duration, and a decrease in plasma glucose level from approximately 100 to 25 per cent was observed at one or two hours after the administration (figure 3). In contrast, the insulin preparation in the absence of surfactant produced slight hypoglycemia at the pH.

The hypoglycemic effects of 50 U./dog of the insulin preparations with pHs ranging from 3.1 to 7.4 were further studied in combination with and without 1 per cent BL-9 (figure 4). In the absence of 1 per cent BL-9, the hypoglycemic effect of the insulin preparation was dependent on its pH—that is, deviation of pH from the isoelectric point of pH 6.0 resulted in enhancement of the effect. In contrast, combination

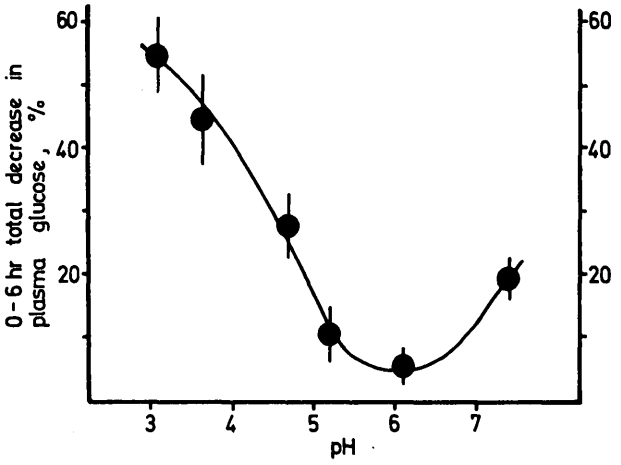


FIG. 2. Effect of pH on the nasal absorption of insulin in dogs. (50 U./dog) The data are expressed as mean ± S.E. (n=4).

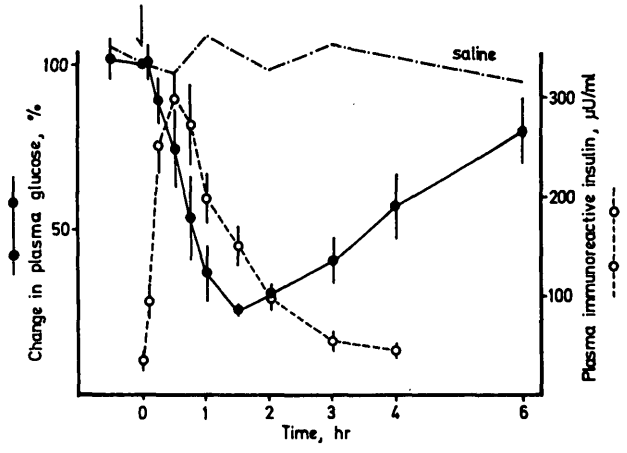


FIG. 1. Changes in plasma glucose (—●—) and plasma immunoreactive insulin (--O--) after intranasal administration of insulin at a dose of 50 U./dog (pH 3.1). The data are expressed as mean ± S.E. (n=5).

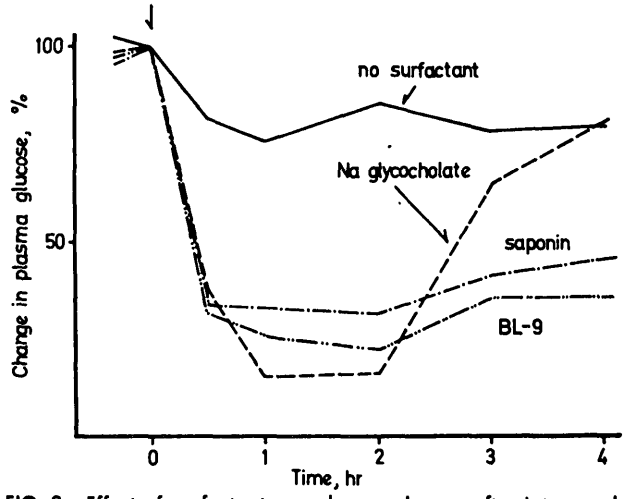


FIG. 3. Effect of surfactants on plasma glucose after intranasal administration of insulin at a dose of 50 U./dog (pH 7.4, surfactant 1 per cent). The data are expressed as the mean of five dogs.

with 1 per cent BL-9 enhanced the hypoglycemic effect irrespective of its pH. Therefore, the enhancement was more markedly observed in the insulin preparation with pH close to the isoelectric point of insulin than in acid or neutral pH.

### *Dose-response Relationship by Intranasal Application of Insulin*

The dose-response curves of the nasally administered insulin preparation with pH 3.1 and that with pH 7.4 in the presence of 1 per cent sodium glycocholate are shown in figure 5 as well as those of the intravenously, intramuscularly, and subcutaneously administered insulin preparations. When log doses of insulin are plotted against the decrement of the plasma glucose level from zero to four hours, the curves for intranasal administration are displaced to the right of those for intravenous, intramuscular, and subcutaneous administrations, although the curves for intranasal and intravenous administrations appear parallel. Even the poorly absorbable insulin preparation at pH 7.4 through the nasal mucosa, when combined with 1 per cent sodium glycocholate, produced hypoglycemia more effectively than the easily absorbable insulin preparation at pH 3.1. The dose of the intranasal application needed to be about three or four times that for intravenous application to produce hypoglycemia of similar magnitude.

## DISCUSSION

Clinical problems in the use of insulin are (1) requirement of parenteral administration, (2) necessity of frequent administration of fast-acting insulin per day, and (3) occurrence of hypoglycemia during sleep or in the fasting condition with treatment by slow-acting insulin. However, insulin preparations for nonparenteral administration have not been available clinically.

Intranasal application of insulin in the anesthetized dogs resulted in the rise of plasma IRI and concomitantly in significant hypoglycemia. This shows that insulin is absorbed from the nasal mucosa into systemic blood as an active form without being subjected to metabolism. However, the nasal absorption of insulin depended on the pH of the preparation. The insulin preparation with the pH ranging from the isoelectric point to neutral induced less hypoglycemia, and the maximum hypoglycemia was found in the insulin preparation at pH 3.1. The smaller amount of hypoglycemia after use of the former preparation appears to derive from (1) difficulty of the effective local absorption due to the low solubility of insulin in water and, consequently, its presence in suspension and (2) the

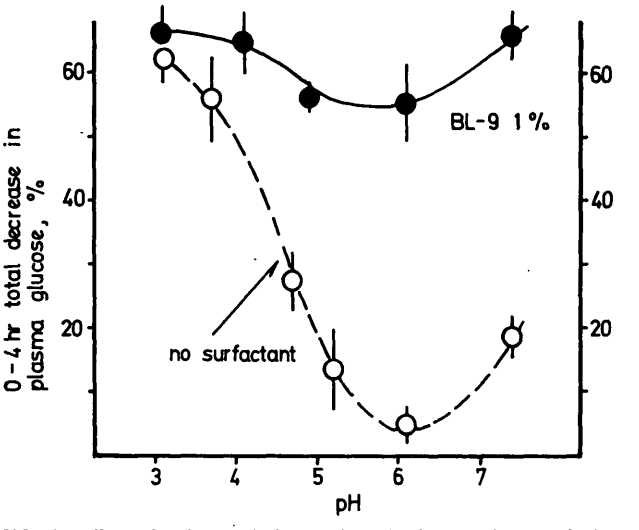


FIG. 4. Effect of polyoxyethylene-9-lauryl ether on the nasal absorption of insulin at a dose of 50 U./dog with various pH. The data are expressed as mean ± S.E. (n=4).

formation of hexomer or octamer at pH close to the isoelectric point, in contrast to the presence as monomer at acid pH.[10] The hypoglycemic effects after intranasal application of insulin preparations were dose-dependent. Furthermore, the dose of intranasal application had to be about three or four times the intravenous dose to produce hypoglycemia of similar magnitude.

On the other hand, since the nasal absorption of insulin from a neutral preparation is not satisfactory, the hormone must be administered together with some agent enhancing nasal absorption. When a surfactant such as saponin, sodium glucocholate, or BL-9 was added to the preparation, the absorption of insulin from the nasal mucosa was enhanced. In this case, the hypoglycemic effect was induced significantly throughout a pH ranging from 3.1 to 7.4.

The mechanism of the enhancing effect on the nasal absorption of insulin by these surfactants is not suffi-

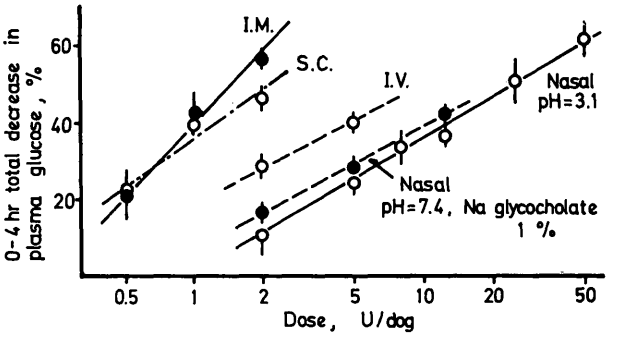


FIG. 5. Dose-response curves after intranasal, intravenous, subcutaneous, and intramuscular administration of insulin in dogs. The data are expressed as mean ± S.E. (n=4 ~ 5).

ciently clear. In previous studies it was shown that the gastrointestinal absorption of highly water-soluble drugs was enhanced by bile acid or nonionic surfactants through increasing the permeability of gastrointestinal mucosa.[11-17] It is suggested that the alteration of the nasal mucosa in the presence of saponin, sodium glycocholate, or BL-9 appears to increase the permeability of the nasal mucosa and to enhance the nasal absorption of a high-molecular polypeptide such as insulin.

To confirm the safety of nasal insulin preparation, toxicity after long-term application should be examined. Therefore, daily administration of sodium glycocholate or BL-9 to the nasal cavity of the rat at a dose of 3 mg./kg. was performed for one month. No significant abnormalities were found, but there was slight change in microvilli when scanning electron microscopy was used to study the morphologic change of the nasal mucosa in the case of BL-9. Sodium glycocholate was less irritative than BL-9 to the nasal mucosa. A more detailed chronic toxicity test would be required for the confirmation of safety of the preparation before clinical application.

In conclusion, the administration of insulin by the nasal route appears to be more effective than other nonparenteral applications proposed earlier and would be useful as a simple and painless dosage form in long-term therapy of diabetes.

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