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Report

Absorption Enhancement of Intranasally Administered Insulin by Sodium Taurodihydrofusidate (STDHF) in Rabbits and Rats

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The enhancement of nasal insulin absorption by sodium taurodihydrofusidate (STDHF) was studied in rabbits and rats. Using identical nasal formulations remarkable interspecies differences were observed. The fusidate derivative at 1% (w/v) enhanced nasal insulin bioavailability from 0.9 to 5.2% and from 0.3 to 18.0% in rabbits and rats, respectively. In both species the insulin formulations with STDHF resulted in strong hypoglycemic responses. Coadministration with the trypsin inhibitor aprotinin tended further to increase insulin bioavailability in rats and decrease insulin bioavailability in rabbits; however, these aprotinin effects were not statistically significant. Addition of the aminopeptidase inhibitor bacitracin to the STDHF containing formulation did not have any effect on insulin bioavailability in rats. Hence, STDHF is a potent enhancer of nasal insulin absorption, probably both by facilitating insulin transport through the nasal mucosa and possibly also by inhibiting enzymatic degradation. Further, interspecies differences and, experimental animal conditions can greatly affect nasal drug absorption.

KEY WORDS: insulin; nasal administration; absorption enhancement; sodium taurodihydrofusidate; rabbits; rats.

INTRODUCTION

Nasal absorption of drugs has received much interest over the past years. Particularly for peptide drugs, such as the antidiabetic polypeptide insulin, the intranasal route of administration has several advantages over parenteral administration, since no injection is required and first-pass elimination is circumvented (1). Nasal delivery of insulin has been investigated in rats (2–4), dogs (5,6), and sheep (7). From these studies it is evident that interspecies differences exist in the amount of intranasally absorbed insulin (2,6). Further difficulties in comparing data from different species arise from the use of different absorption enhancers, different types of insulin, and different concentrations of insulin and additives in the nasal formulations. In addition, nasal absorption studies of sodium cromoglycate in rats have shown that experimental animal conditions can also affect the nasal absorption properties of drugs (8). In order to identify the interspecies problems encountered, we performed experiments in rabbits and rats, using nasal formulations with identical insulin and additive concentrations.

The present study was focused on the absorption promoting effect of sodium taurodihydrofusidate (STDHF) on intranasally administered insulin in rabbits and rats, because STDHF is an excellent and biocompatible enhancer of insulin transport across the nasal mucosa of sheep (7).

MATERIALS AND METHODS

Chemicals

Solutions of human insulin (100 IU/ml; Humulin), obtained from Eli Lilly (Giessen, The Netherlands), were used for all intravenous and nasal formulations. Sodium tauro-24,25-dihydrofusidate (STDHF) and aprotinin were gifts from CalBio (Mountain View, Calif.) and Bayer (Leverkusen, F.G.R.), respectively. Bacitracin was obtained from Sigma (Amsterdam, The Netherlands). Hypnorm was from Janssen Pharmaceutica (Goirle, The Netherlands).

Insulin Formulations

All formulations were prepared aseptically by diluting appropriate amounts of the human insulin solutions with stock solutions of the enhancers in 0.9% saline. Insulin concentrations in all nasal formulations were 80 IU/ml, whereas STDHF, aprotinin, and bacitracin concentrations were 1%, 10^4 kIU/ml, and 1%, respectively. For intravenous administration to rabbits a 20-IU/ml human insulin solution was prepared by diluting with 0.9% saline, whereas for rats a 10-IU/ml solution was used.

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Nasal Absorption Studies in Rabbits

Five New Zealand rabbits, weighing approximately 4 kg, were given 0.7 ml of Hypnorm intramuscularly to prevent sneezing while formulations were instilled intranasally. Nasal insulin formulations (0.1 ml, corresponding to 8 IU insulin) were instilled through the nares unilaterally using a syringe connected with a PVC canula. An intravenous bolus injection (0.1 ml) of 2 IU human insulin in the ear vein and placebo (intranasal 0.9% saline) was given to determine absolute bioavailabilities of the nasal insulin formulations. Venous blood samples were taken from an ear vein at regular time intervals. Nasal formulations containing either no enhancer, STDHF, or a combination of STDHF and aprotinin were administered in a random order to each of the five rabbits. Subsequent administrations were given after a washout period of at least 1 week.

Nasal Absorption Studies in Rats

Male Wistar rats, weighing 175–225 g, were anaesthetized with Hypnorm (0.1 ml/100 g) intramuscularly, and additional Hypnorm (0.05 ml/100 g) was given usually 75 and 165 min after the first injection. In order to facilitate nasal administrations and to prevent peroral absorption, the trachea was canulated and the esophagus was tied to this canula. Comparable surgical procedures have been described by other investigators (8,9).

Animals were kept lying on their back on thermostated rugs (37°C) during the experiment. Nasal formulations (5 μl, corresponding to 0.4 IU insulin) were instilled unilaterally through the nares 105 min after the first Hypnorm injection using PVC tubing affixed to a microliter syringe. Blood samples were taken from a canulated femoral artery at regular time intervals. Nasal insulin formulations were administered containing either no enhancer, STDHF alone, or combinations of STDHF and aprotinin or bacitracin.

Intravenous administration of 0.1 IU (10 μl) human insulin and placebo (intranasal 0.9% saline) were given to determine absolute bioavailabilities. For intravenous administrations the trachea canula was omitted and a femoral vein was canulated.

Analytical Procedures

Serum levels of immunoreactive human insulin were measured using a commercially available radioimmunoassay kit (Insulin RIA 100, Pharmacia Diagnostics, Uppsala, Sweden). The antiserum shows 100% cross-reactivity with bovine and porcine insulin, whereas the cross-reactivity with C peptide is ≤0.2%. The detection limit is ≤2 μU insulin/ml serum.

Blood glucose concentrations were determined using Haemo-Glukotest sticks in combination with a Reflolux II reflectance meter (Boehringer Mannheim, Almere, The Netherlands).

Data Analysis

The areas under the individual serum/blood concentration–time curves for both insuln and glucose were calculated using the linear trapezoidal rule. In the rabbits each animal was its own control and bioavailabilities were determined for each individual animal. In the rat experiments bioavailabilities were calculated using the mean AUC values. For statistical evaluation of the results the one-tailed Student's t test was used. Differences were assigned to be statistically significant for values of $P < .05$.

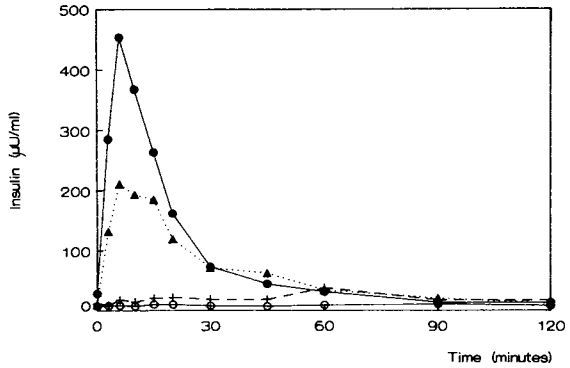

Fig. 1. Mean serum concentrations of insulin after nasal administration of 8 IU insulin to rabbits. ○, 0.9% saline; +, insulin without enhancer; ●, insulin with STDHF; ▲, insulin with STDHF/aprotinin.

RESULTS

In rabbits nasal administration of the insulin formulations containing 1% (w/v) of the enhancer STDHF gives significantly higher insulin absorption and lower glucose levels than the formulations without enhancer (Figs. 1 and 2). Relative to an intravenous injection of human insulin, an absolute bioavailability of 5.2% can be calculated for the nasal formulation containing 1% STDHF, which is 5.8 times higher than that of the nasal formulation without enhancer (Table I). The addition of the trypsin inhibitor aprotinin does not further enhance insulin absorption; insulin and glucose AUCs even tend to decrease absorption, although this effect is not statistically significant (Table I).

In the rat model nasal administration of human insulin without enhancer does not result in elevated serum levels of immunoreactive insulin as compared to placebo administration. Insulin absorption is greatly enhanced and blood glu-

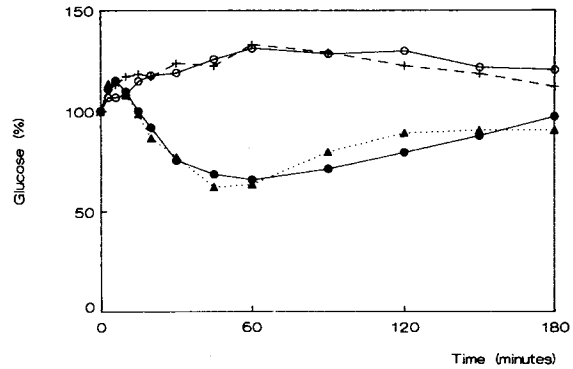

Fig. 2. Mean blood glucose concentrations after nasal administration of 8 IU insulin to rabbits. ○, 0.9% saline; +, insulin without enhancer; ●, insulin with STDHF; ▲, insulin with STDHF/aprotinin.

Table I. AUC Values of Insulin and Glucose and Bioavailabilities (F) of Insulin After i.v. and Intranasal (i.n.) Administration of Various Doses of Insulin With or Without STDHF (1%, w/v), Aprotinin (Apro; 10^4 kIU/ml), and Bacitracin (Bac; 1%, w/v) in Rabbits and Rats[a]

	Dose (IU)	Route	Additive	AUC insulin ($\mu U \cdot ml^{-1} \cdot min$)	AUC glucose (% of initial values $\times 10^3$)	F insulin (%)	(N)
Rabbits	2	i.v.	—	48,535 ± 15,666	10.8 ± 2.3	100	(5)
	0	i.n.	—	1,073 ± 208	22.4 ± 4.5	0	(5)
	8	i.n.	—	2,562 ± 1,816	21.7 ± 5.3	0.9 ± 1.0	(5)
	8	i.n.	STDHF	9,542 ± 3,844**	14.8 ± 2.1*	5.2 ± 3.5	(5)
	8	i.n.	STDHF/Apro	7,189 ± 4,708*	15.1 ± 1.3*	3.7 ± 2.9	(5)
Rats	0.1	i.v.	—	10,472 ± 3,336	11.3 ± 1.6	100	(6)
	0	i.n.	—	854 ± 301	14.2 ± 2.0	0	(3)
	0.4	i.n.	—	963 ± 380	14.0 ± 0.6	0.3 ± 1.0	(4)
	0.4	i.n.	STDHF	7,778 ± 2,846**	11.1 ± 1.2**	18.0 ± 7.4	(6)
	0.4	i.n.	STDHF/Apro	9,411 ± 3,314**	11.8 ± 1.6**	22.2 ± 8.6	(6)
	0.4	i.n.	STDHF/Bac	7,527 ± 4,574**	11.9 ± 1.9*	17.3 ± 11.9	(6)

[a] All values are the mean ± SD for the number of animals given in parentheses (N). AUC represents the area under the blood/serum concentration–time curve, as determined to 2 hr for insulin, to 2 hr for glucose in rats, and to 3 hr for glucose in rabbits.

* Significantly different from nasal insulin formulation without additives: $P < 0.05$.

** Significantly different from nasal insulin formulation without additives: $P < 0.01$.

cose decreased by the coadministration of 1% STDHF in the nasal insulin formulations (Figs. 3 and 4). Relative to an intravenous bolus injection of 0.1 IU human insulin, an absolute bioavailability of 18% can be calculated (Table I). The addition of aprotinin to the formulations leads to enhanced insulin levels and insulin AUCs (Fig. 3 and Table I). However, this apparent rise in insulin absorption is not statistically significant. Coadministration of the aminopeptidase inhibitor bacitracin to the nasal insulin formulation has no additional effect on insulin or glucose levels (Table I).

DISCUSSION

In both rabbits and rats the fusidate derivative STDHF greatly enhanced insulin absorption, resulting in increased serum levels of immunoreactive insulin and reduced blood glucose levels. Addition of the proteolytic enzyme inhibitors aprotinin or bacitracin to the nasal formulations did not lead to a further increase in insulin absorption. Therefore, the metabolic degradation of insulin is probably negligible in nasal formulations already containing STDHF. This is in accordance with the previously reported inhibiting properties of STDHF on peptidase activities in nasal mucosal homogenates (10). We have not studied the effects of aprotinin and bacitracin alone on nasal insulin absorption, because no significant nasal absorption of large molecules such as insulin can be expected from inhibition of peptidase activities alone. Only a slight absorption promoting effect of aprotinin on nasal insulin absorption has been demonstrated in rats. Aprotinin in combination with the absorption promoter laureth-9, however, did not result in an enhancement of nasal insulin absorption over laureth-9 alone (11). The presented remarkable differences between rabbits and rats in nasal bioavailability of the STDHF containing insulin formulations may be contributed to interspecies differences. It is known that nasal insulin absorption without additives is higher in dogs than in rats. This interspecies difference appears to decrease after addition of absorption enhancers to the formulations (2,5). Our data show that intranasally administered insulin without enhancer is absorbed to a larger extent

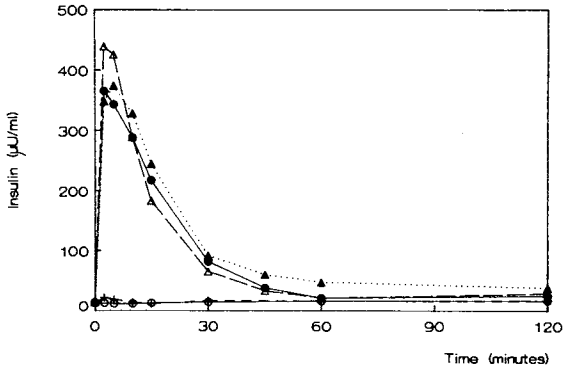

Fig. 3. Mean serum concentrations of insulin after nasal administration of 0.4 IU insulin to rats. ○, 0.9% saline; +, insulin without enhancer; ●, insulin with STDHF; ▲, insulin with STDHF/aprotinin; △, insulin with STDHF/bacitracin.

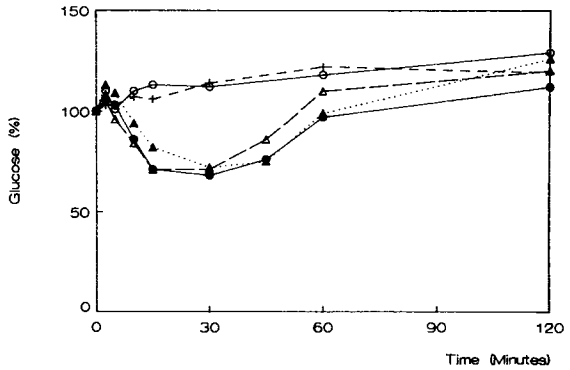

Fig. 4. Mean blood glucose concentrations after nasal administration of 0.4 IU insulin to rats. ○, 0.9% saline; +, insulin without enhancer; ●, insulin with STDHF; ▲, insulin with STDHF/aprotinin; △, insulin with STDHF/bacitracin.

in rabbits than in rats, whereas with enhancer the reverse is observed (Table I).

Experimental animal conditions may be important as well. For instance, in studies on the nasal absorption of sodium cromoglycate in rats (8) it was found that mucociliary clearance or drainage was inhibited when the animals were lying on their backs. Approximately 20% of the intranasally instilled dose, however, was recovered in a tracheal canula when the animals were on their bellies with unsealed nasopalatine and nostrils (8). In the rabbits used in our experiments nasal functioning can be assumed to be intact with normal mucociliary clearance removing intranasally instilled formulations from the nasal cavity. In the presented rat model nasal functioning may be impaired: the animals stop nasal breathing after the operation, their esophagus is tied off, and they are maintained on their backs during the experimental procedure. This impairment of mucociliary clearance in the rat model may result in longer nasal residence times for the drug formulations as compared to the rabbits. This in turn will allow a larger fraction of the nasally administered insulin dose to penetrate across the nasal mucosa.

Bioadhesive microspheres can also result in prolonged nasal residence times, leading to enhanced absorption of poorly absorbed hydrophilic drugs as was shown by for the antibiotic compound gentamycin (12) and for insulin (13). The nasal formulations may also contact different anatomical structures of the nasal mucosa in rabbits and rats. In rabbits in suppine position most of the formulation can be expected to spread over the bottom of the nasal cavity. In rats lying on their back it is more likely that nasally administered drugs have to pass through the upper part of the nasal cavity. It is well-known that histological differences exist between these regions (14). Most of the nasal cavity is lined with respiratory epithelium comprised of ciliated cuboidal and columnar cells and goblet cells. The olfactory epithelium at the roof of the nasal cavity is composed of a pseudostratified neuroepithelium. The absence of nasal cilia and mucociliary clearance from the upper region is probably an important factor in the increased effectiveness of nasal sprays as compared to drops, again resulting from an increase in nasal residence times (15–17).

In conclusion, the nasal absorption of insulin is remarkably promoted by the biocompatible fusidate analogue STDHF both in rabbits and in rats. The extent of insulin absorption, however, varies among species and experimental conditions.

REFERENCES

1. Y. W. Chien and S. F. Chang. In Y. W. Chien (ed.), *Transnasal Systemic Medications,* Elsevier, Amsterdam, 1985, pp. 1–99.
2. S. Hirai, T. Yashiki, and H. Mima. *Int. J. Pharm.* 9:165–172 (1981).
3. S. Hirai, T. Yashiki, and H. Mima. *Int. J. Pharm.* 9:173–184 (1981).
4. K. Morimoto, K. Morisaka, and A. Kamada. *J. Pharm. Pharmacol.* 37:134–136 (1984).
5. S. Hirai, T. Ikenaga, and T. Matsuzawa. *Diabetes* 27:296–299 (1978).
6. T. Nagai, Y. Nishimoto, N. Nambu, Y. Suzuki, and K. Sekine. *J. Control. Rel.* 1:15–22 (1984).
7. J. P. Longenecker, A. C. Moses, J. S. Flier, R. D. Silver, M. C. Carey, and E. J. Dubovi. *J. Pharm. Sci.* 76:351–355 (1987).
8. A. N. Fisher, K. Brown, S. S. Davis, G. D. Parr, and D. A. Smith. *J. Pharm. Pharmacol.* 37:38–41 (1985).
9. S. Hirai, T. Yashiki, T. Matsuzawa, and H. Mima. *Int. J. Pharm.* 7:317–325 (1981).
10. V. H. L. Lee, D. Gallardo, and J. P. Longenecker. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* 14:55–56 (1987).
11. B. J. Aungst and N. J. Rogers. *Pharm. Res.* 5:305–308 (1988).
12. L. Illum, N. Farraj, H. Critchley, and S. S. Davis. *Int. J. Pharm.* 46:261–265 (1988).
13. E. Björk and P. Edman. *Int. J. Pharm.* 47:233–238 (1988).
14. J. T. Young. In C. S. Barrow (ed.). *Toxicology of the Nasal Passages,* Hemisphere, Washington, D.C., 1986, pp. 27–36.
15. J. G. Hardy, S. W. Lee, and C. G. Wilson. *J. Pharm. Pharmacol.* 37:294–297 (1985).
16. K. S. E. Su. *Pharm. Int.* 1:8–11 (1986).
17. A. S. Harris, J. M. Nilsson, Z. G. Wagner, and U. Alkner. *J. Pharm. Sci.* 75:1085–1088 (1986).