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Report

Absorption Enhancement of Rectally Infused Insulin by Sodium Tauro-24,25-Dihydrofusidate (STDHF) in Rats

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The bile salt derivative sodium tauro-24,25-dihydrofusidate (STDHF) has been reported to promote nasal absorption of insulin. In the present study the effect of STDHF on rectal insulin absorption was investigated in rats. At concentrations of 1 and 4% (w/v) it enhanced insulin bioavailability from 0.2 ± 0.2 (control) to 4.2 ± 3.2 and 6.7 ± 2.1%, respectively, as assessed by radioimmunoassay. Insulin preparations with STDHF reduced blood glucose concentrations considerably in a concentration-dependent way. Coadministration of STDHF with Na_2EDTA (0.25%, w/v) tended to increase further insulin bioavailability and hypoglycemic response. Varying the site of rectal administration did not influence these parameters.

KEY WORDS: insulin; absorption; sodium tauro-24,25-dihydrofusidate; rectum; rats.

INTRODUCTION

Daily parenteral injections of insulin represent a potential reason for poor acceptability of insulin treatment. Therefore, the development of clinically effective, nonparenteral routes of insulin delivery has been the objective of many research efforts. Insulin has been coadministered with absorption enhancing agents or enzyme inhibitors, in order to promote passage of epithelial barriers and to reduce degradation. For instance, the rectal absorption of insulin has been reported to be promoted by polyoxyethylene ethers (1) and various other surfactants (2), enamines (3), salicylate (4), bile salts (5), and enzyme inhibitors (6) in rats, rabbits, and dogs. In man, rectal insulin absorption was shown to be promoted by salicylate and an enamine (7) and by polyoxyethylene-9-lauryl ether (8). Attempts have been made to enhance insulin absorption by the nasal route in man by coadministration of 1% (w/v) sodium glycocholate (9) and sodium deoxycholate (10). However, bile salts were shown to be ciliotoxic (11), and toxic effects on chronic intranasal administration with deoxycholate proved to be considerable (12), implying the need for other, less irritating, absorption promoters.

Recently, Longenecker *et al.* reported a promoting effect of the fusidic acid derivative, sodium tauro-24,25-dihydrofusidate (STDHF), on nasal insulin absorption in sheep, resulting in a bioavailability of maximally 16%, without causing damage to the nasal epithelium (13). In man, 1% STDHF increased nasal insulin bioavailability to 11% (14). As rectal administration of insulin with various absorption promoters elicited a comparable or stronger hypoglycemic response in rats, compared with nasal administration (15,16), it was decided to investigate the effects of STDHF on rectal insulin absorption in rats. Because portal administration has been suggested to be important in eliciting the hypoglycemic effect of insulin (17), insulin was delivered close to the anus as well as 1 cm from the anus. The latter site is likely to result in a higher proportion of the absorbed drug going directly to the liver (18). As insulin is susceptible to degradation by mucosal proteinases (19), it is conceivable that enzymatic degradation before and during absorption contributes to the low bioavailability of insulin, when administered in the gastrointestinal tract. Therefore, the effect of coadministration of the metalloproteinase inhibitor EDTA (20) was also evaluated, at a concentration which was demonstrated to reduce degradation of the neuropeptide desenkephalin-γ-endorphin in the rectal lumen (21).

MATERIALS AND METHODS

Chemicals

Human insulin (25 U/mg) was obtained as a gift from Diosynth (Oss, The Netherlands). Sodium tauro-24,25-dihydrofusidate.3aq was kindly supplied by California Biotechnology, Inc. (Mt. View, CA). Human albumin (fraction V) was obtained from Sigma Chemical Co. (St. Louis, MO). $Na_2EDTA.2aq$ was purchased from J. T. Baker Chemicals B.V. (Deventer, The Netherlands). All other chemicals used were of analytical grade.

Animals

Male Wistar rats of laboratory breed, weighing 160 to

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190 g, were used. Food was withdrawn 16 hr prior to surgery, and water was provided *ad libitum* throughout the experiment. Each rat participated in one experiment only.

Drug Solutions

For i.v. administration solutions of insulin (20 and 40 μg/ml) in saline were used, whereas for rectal administration solutions of 160 μg/ml containing 0 to 4% (w/v) STDHF with or without 0.25% (w/v) Na_2EDTA were applied. For studying a potential interaction of rectally delivered STDHF with the elimination kinetics of i.v.-infused insulin, an aqueous solution of 1% (w/v) STDHF was infused rectally. To all solutions 0.5% (w/v) human albumin was added, which was demonstrated to reduce considerably adsorption of insulin to polyvinylchloride cannulae, resulting in a negligible loss of insulin by adsorption.

Drug Administration and Blood Sampling

Drug administration and blood sampling were performed as described previously (22). In short, 200 μl of drug solution was infused i.v. or rectally in 32 min. Rectal administration was carried out at a distance of 2 or 10 mm from the anus. During and after drug infusion, arterial blood samples of 50 μl were collected and plasma was obtained by centrifugation.

Assays of Insulin and Glucose

Plasma samples of 25 μl were diluted in 100 μl of assay buffer, containing sodium barbitone (4.12 g/liter), Na_2EDTA (3.72 g/liter), L-cystine (16 mg/liter), sodium chloride (8.12 g/liter), and human albumin (5 g/liter). Insulin was measured in the diluted plasma samples using a radioimmunoassay (Insulin RIA 100, Pharmacia Nederland B.V., Woerden, The Netherlands). Linearization of the calibration curves was performed by the "logit-log" method (23). As weighting factor was used $(1 - B/B_0) \times B/B_0$, B and B_0 representing counts in the sample and in the zero standard, respectively, both corrected for nonspecific binding.

Glucose levels were measured in arterial blood samples using the Reflolux II M system (Boehringer Mannheim B.V., Almere, The Netherlands).

Data Analysis

Areas under the individual plasma insulin concentration–time curves from $t = 0$ to the last sampling point (AUC) were calculated using the linear-logarithmic trapezoidal rule. Systemic insulin clearances were calculated as D/AUC, with D referring to the i.v. dose.

Blood glucose concentrations, normalized to percentage of the initial concentration, were plotted against time, and areas under the normalized concentration–time curves were calculated using the linear trapezoidal rule. The hypoglycemic response was calculated as the sum of the areas above (positive value) and below (negative value) the 100% level (16).

The Wilcoxon rank sum test was used for statistical evaluation of the results, maintaining a comparisonwise error rate of 0.05. Results of infusions at a distance of 2 mm from the anus were evaluated, using rectal delivery without enhancer as control. The delivery with 1% (w/v) STDHF at 2 mm was used as control for testing the effects of 4% (w/v) STDHF, of EDTA, and of delivery at 10 mm.

RESULTS

Intravenous infusion of 4 μg of insulin resulted in a mean systemic clearance of 15 ± 9 ml/min ($n = 6$). A dose of 8 μg resulted in dose-corrected AUC values which were comparable with those of the 4-μg infusion (Table I) and in a clearance value of 11 ± 2 ml/min ($n = 3$), indicating linearity of insulin elimination kinetics in this i.v. dose range. Upon i.v. infusion of 4 μg of insulin with concurrent rectal infusion of 1% (w/v) STDHF, AUC values and hypoglycemic responses were comparable to those obtained without delivery of STDHF (Table I). A mean insulin clearance of 13 ± 2 ml/min ($n = 3$) was calculated, which excludes an interaction of rectally delivered STDHF with insulin elimination kinetics.

Rectal infusion of insulin without enhancer resulted in very low insulin plasma concentrations and did not elicit any hypoglycemic response (Fig. 1). Coadministration of 1% (w/v) STDHF appreciably increased insulin plasma concentrations and reduced blood glucose concentrations (Fig. 1). Insulin bioavailability was significantly increased, from 0.2 ±

Table I. Dose-Corrected AUC Values, Bioavailabilities (F), and Hypoglycemic Responses (±SD) Observed on i.v. and Rectal (i.r.) Infusion of Various Doses of Insulin with or Without STDHF (1 or 4%, w/v) and $Na_2EDTA.2aq$ (0.25%, w/v), i.r.2 and i.r.10 Referring to Delivery at 2 and 10 mm from the Anus, Respectively

Insulin dose (μg)	Route	Conc. (%, w/v) STDHF	Conc. (%, w/v) EDTA	AUC/D (10^{-3} min/ml)	F (%)	Hypoglycemic response (100% · min)	n
4	i.v.	—	—	80 ± 27	100	−30 ± 8	6
4	i.v.	1	0	80 ± 12	100	−34 ± 5	3
8	i.v.	—	—	95 ± 15	100	−38 ± 3	3
32	i.r.2	0	0	0.2 ± 0.1	0.2 ± 0.2	5 ± 7	6
32	i.r.2	1	0	3.3 ± 2.6*	4.2 ± 3.2*	−15 ± 9*	6
32	i.r.10	1	0	3.2 ± 2.4	4.0 ± 3.1	−8 ± 12	6
32	i.r.2	1	0.25	4.2 ± 2.0*	5.2 ± 2.7*	−22 ± 6*	6
32	i.r.2	4	0	5.4 ± 1.7*	6.7 ± 2.1*	−25 ± 3*,**	6

* Significantly different from rectal infusion without STDHF ($P < 0.05$, Wilcoxon rank sum test).

** Significantly different from infusion at 2 mm with 1% (w/v) STDHF ($P < 0.05$, Wilcoxon rank sum test).

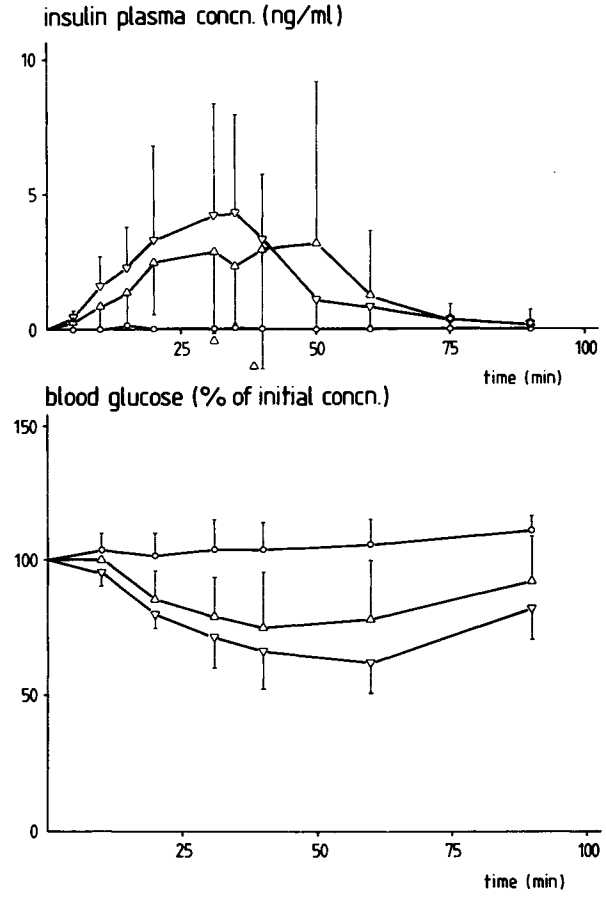

Fig. 1. Mean insulin plasma concentrations (upper) and hypoglycemic responses (lower) (±SD) observed during and after rectal infusion for 32 min of 32 μg of insulin without enhancer (○), with 1% (w/v) STDHF (△), and with 1% (w/v) STDHF and 0.25% (w/v) $Na_2EDTA.2aq$ (▽) in rats, at a distance of 2 mm from the anus.

0.2 to 4.2 ± 3.2% (Table I). The addition of EDTA resulted in comparable values, although plasma concentrations (Fig. 1), insulin bioavailabilities, and hypoglycemic responses (Table I) tended to increase. When the STDHF concentration was increased from 1 to 4% (w/v), the mean insulin bioavailability demonstrated a slight but insignificant increase, whereas the hypoglycemic response was significantly greater (Table I). Comparable insulin bioavailabilities and hypoglycemic responses were obtained when insulin was infused rectally with STDHF at a distance of 2 or 10 mm from the anus (Table I).

DISCUSSION

STDHF at concentrations of 1 and 4% (w/v) proved to increase rectal insulin bioavailability to a considerable extent (Fig. 1, Table I). These concentrations correspond with the range resulting in enhancement of nasal insulin absorption (13) and in enhanced rectal absorption of cefoxitin and desglycinamide arginine vasopressin in rats (24). Several other compounds, e.g., bile salts and surfactants, are effective as enhancers of intestinal drug uptake in comparable concentrations, as reviewed recently (25).

The tendency of increasing insulin bioavailability and hypoglycemic response on coadministration with EDTA and STDHF, compared with STDHF alone (Fig. 1, Table I), suggests a weak proteolysis inhibiting effect of EDTA. This observation indicates that a further exploration of the influence of enzyme inhibitors on intestinal peptide bioavailability is justified. On the other hand, it cannot be excluded that a paracellular or transcellular absorption enhancing effect of EDTA is involved in this observation (25).

As insulin infusion close to the anus did not increase bioavailability or reduce the hypoglycemic effect, compared with delivery 10 mm deep (Table I), both delivery sites seem to result in comparable amounts of insulin reaching the liver. In contrast, infusion of the high-clearance drug lidocaine close to the anus has been reported to reduce the amount of drug passing directly to the liver through the portal system (18). These different results between insulin and lidocaine may be explained by preferential lymphatic absorption of insulin, which has been suggested previously for insulin when rectally delivered with 5-methoxysalicylate (17). Additionally, it is conceivable that on delivery close to the anus, insulin may be partly absorbed at a deeper site of the rectum, because of spreading of the solution infused. This spreading behavior has been demonstrated previously (22).

Portal delivery of insulin has been suggested to be important for an efficient hypoglycemic response (17). However, in the present study the amount of insulin rectally absorbed does not elicit a stronger hypoglycemic response, compared to the amount infused i.v. (Table I). It is conceivable that this observation is also based on the predominant lymphatic absorption of insulin, resulting in a relatively small amount of drug reaching the liver directly (8,17).

The results of this study demonstrate that rectal administration of insulin with STDHF elicits an important hypoglycemic response, which is caused by enhanced insulin bioavailability. Future studies should be directed to the safety aspects of rectally delivered STDHF and to optimization of the formulation; in this respect concentrations of both absorption promoter and of enzyme inhibitor may be important variables. Results obtained in this study indicate that a concentration of 1% STDHF is a promising starting point for further studies.

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