Absorption Enhancing Effect of Cyclodextrins on Intranasally Administered Insulin in Rats

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The absorption enhancing effect of α-, β-, and γ-cyclodextrin (CD), dimethyl-β-cyclodextrin (DMβCD), and hydroxypropyl-β-cyclodextrin (HPβCD) on intranasally administered insulin was investigated in rats. Coadministration of 5% (w/v) DMβCD to the insulin solution resulted in a high bioavailability, 108.9 ± 36.4% (mean ± SD, $n = 6$), compared to i.v. administration, and a strong decrease in blood glucose levels, to 25% of their initial values. Coadministration of 5% α-CD gave rise to an insulin bioavailability of 27.7 ± 11.5% (mean ± SD, $n = 6$) and a decrease in blood glucose to 50% of its initial value. The rate of insulin absorption and the concomitant hypoglycemic response were delayed for the α-CD-containing solution as compared to the DMβCD preparation. The other CDs, HPβCD (5%), β-CD (1.8%), and γ-CD (5%), did not have significant effects on nasal insulin absorption. DMβCD at a concentration of 5% (w/v) induces ciliostasis as measured on chicken embryo tracheal tissue *in vitro*, but this effect is reversible. In conclusion, DMβCD is a potent enhancer of nasal insulin absorption in rats.

KEY WORDS: cyclodextrins; insulin; nasal administration; rats; ciliary movement.

INTRODUCTION

A limiting factor in the therapeutic use of peptide and protein drugs is the lack of appropriate delivery systems. Peptides and proteins are scarcely absorbed following oral delivery as a result of enzymatic degradation in the gastrointestinal tract and poor transport characteristics, and they are therefore administered parenterally (1,2). Parenteral injection therapy, however, has serious drawbacks in chronic therapy, whereas the nasal route is an attractive alternative to parenteral injection because of the rich vascularization, ease of administration, and circumvention of first-pass metabolism (3). Nevertheless, the bioavailability of intranasally administered peptides and proteins, including insulin, may be low because of their high molecular weight and hydrophilicity. Nasal insulin bioavailability can be improved by coadministration of absorption enhancers, such as surfactants (4), bile salts (5), fusidate derivatives (6,7), medium-chain fatty acids (8), and phospholipids (9). Many of these compounds, however, are harmful to the nasal epithelial membranes (10) and interfere with the nasal ciliary movement (11), thus being unsuitable for long-term therapy of intranasally administered insulin.

Recently, we have shown that coadministration of dimethyl-β-cyclodextrin results in a considerable increase in the nasal bioavailability of the female steroid hormones estradiol and progesterone in rabbits and rats (12,13). These nasal formulations exert only minor effects on nasal ciliary movement as measured *in vitro*. Cyclodextrins (CDs) can incorporate lipophilic drugs such as estradiol and progesterone by the formation of inclusion complexes, resulting in an increased water solubility and increased nasal absorption of the complexed drugs (12–14). Besides the described solubilizing effect of CDs on lipophilic drugs, these compounds may also exert direct effects on the epithelial membranes (14,15), thereby facilitating the nasal absorption of high molecular weight, hydrophilic peptides and proteins. Improvement of nasal insulin absorption by underivatized CDs has been reported (16). The aim of the present study was to investigate the absorption promoting effect of various CDs and, especially, their derivatives on intranasally administered insulin in rats and to explore their influence on the ciliary movement *in vitro*.

MATERIALS AND METHODS

Chemicals

Human insulin powder (20 IU/mg) was a gift from Diosynth (Oss, The Netherlands). α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), dimethyl-β-cyclodextrin (DMβCD), and Hypnorm were from Janssen Pharmaceutica (Goirle, The Netherlands), whereas hydroxypropyl-β-cyclodextrin (HPβCD) was from Avebe (Foxhol, The Netherlands).

Formulations

Insulin solutions were prepared by dissolving human insulin powder in 5 m*M* HCl in 0.9% saline and were subsequently neutralized with 0.1 *M* NaOH to insulin concentrations of 1 mg/ml. For nasal application, various cyclodextrins were added to the insulin solutions. The final concentrations of DMβCD, α-CD, γ-CD, and HPβCD were 5% (w/v), whereas the final concentration of β-CD was 1.8% (w/v), being the maximum amount of β-CD which can be dissolved in aqueous solutions. For intravenous (i.v.) administration, the insulin solution was diluted eight times with 0.9% saline to a concentration of 125 μg/ml. Placebo formulations contained the same concentrations of cyclodextrins in 0.9% saline without insulin.

Nasal Absorption Studies

Nasal absorption studies were performed as reported earlier (6). Briefly, male Wistar rats of approximately 200 g were fasted overnight with tap water freely available. They were anesthetized with intramuscularly injected Hypnorm (1 ml/kg). The arteria femoralis was canulated to take blood samples. The trachea was canulated with a Teflon tube to prevent nose respiration, and the esophagus was tied to this cannula to prevent peroral absorption. For intravenous (i.v.) administration the femoral vein was also canulated. The na-

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sal preparations were administered unilaterally through the nares using PVC tubing connected to a microliter syringe. A total volume of 20 μl was administered for intranasal (i.n.) administration as well as i.v. administration, corresponding to 0.4 IU (20 μg) and 0.05 IU (2.5 μg) insulin, respectively. Blood samples (300 μl/sample) were taken at regular time intervals. The samples were centrifuged to obtain serum and subsequently stored at −20°C until analysis.

Analytical Procedures

Blood glucose levels were measured immediately after blood sampling using Haemo-Glucotest sticks in combination with a Reflolux II reflectance meter (Boehringer Mannheim, Almere, The Netherlands).

Serum insulin concentrations were determined with a commercially available radioimmunoassay kit (insulin RIA 100, Pharmacia Diagnostics, Woerden, The Netherlands). The antiserum showed 100% cross-reactivity with bovine and porcine insulin and 0.2% with the C peptide. The limit of detection was ≤2 μU/ml serum.

Data Analysis

The areas under the serum/blood concentration–time curves (AUC) for insulin as well as for glucose were calculated using the linear trapezoidal rule. AUC values were corrected for endogenous basal levels of insulin by subtraction of the mean AUCs obtained after placebo administration. Nasal bioavailabilities (F) were calculated according to the formula $[(AUC_{i.n.} - AUC_{pl})/(AUC_{i.v.} - AUC_{pl})] \times 100\%$. For statistical evaluation of the AUC values the Wilcoxon rank test was used. Differences were assigned to be significant for values of $P \leq 5\%$.

Ciliary Beat Frequency Measurements

Ciliary beat frequency (CBF) measurements were performed on ciliated tissue of chicken embryo trachea with a photoelectric registration device as described previously (17). Briefly, a light beam is transmitted through the moving cilia, and after magnification by a microscope the flickering light is projected on a photocell. The electrical signal, generated by this photocell, is visualized with an oscilloscope and the frequency of this signal is estimated electronically and displayed. The CDs investigated were dissolved in Locke–Ringer (LR); the final concentrations of DMβCD, α-CD, γ-CD, and HPβCD were 5% (w/v), whereas the final concentration of β-CD was 1.8% (w/v). Control experiments (blank) were performed in pure LR. All experiments were performed at 30°C. To investigate possible reversibility of the CBF, after the measurement was done at 20 min, the ciliated tissue was replaced in pure LR. Since insulin itself has no effect on the ciliary movement, cyclodextrin solutions at concentrations as used in the nasal formulations were made in LR in the absence of insulin. CBF data were calculated as percentages of the initial values, the latter being 100% (18).

RESULTS

Insulin Absorption Studies

Basal serum insulin levels following placebo administration ranged between 6 and 40 μU/ml during the 2 hr of the experiments. Of the cyclodextrins investigated, DMβCD appears to be the most potent enhancer of nasal insulin absorption in rats. Nasal instillation of insulin with 5% (w/v) DMβCD gave rise to remarkable increases in serum insulin concentrations, with peak values of 740 ± 160 μU/ml at 10 min (Fig. 1), and to concomitant decreases in blood glucose levels, reaching minimal values of 25% of the initial concentrations at 60 min and then remaining fairly constant until the end of the experiment (Fig. 2). Insulin absorption was also enhanced, and blood glucose decreased by coadministration of 5% α-CD. The effects of α-CD were not as strong as those observed with DMβCD. The rate of insulin absorption and the resulting hypoglycemic response were significantly delayed for the α-CD-containing formulations in comparison with the DMβCD preparations. The addition of 5% α-CD gave rise to a maximum insulin level of 115 ± 42 μU/ml at 30 min and resulted in a decrease in blood glucose to 50% of its initial value at 120 min (Figs. 1 and 2). The addition of β-CD (1.8%), HPβCD (5%), and γ-CD (5%) to the nasal insulin formulations did not effect the insulin and glucose concentrations (Table I). Relative to an intravenous injection of 2.5

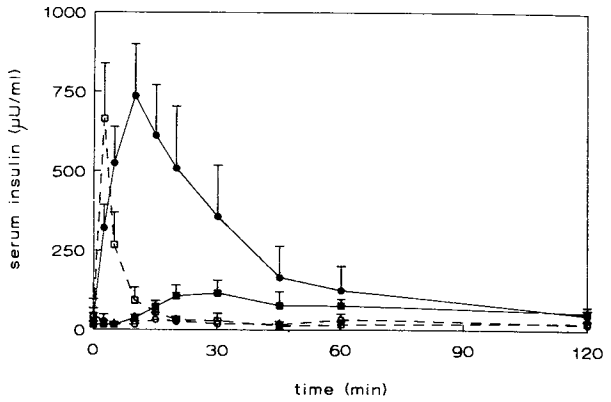

Fig. 1. Mean serum insulin concentrations after intravenous administration of 0.05 IU insulin (□), nasal administration of placebo (○), and nasal administration of 0.4 IU insulin with 5% DMβCD (●) and 5% α-CD (■). Bars represent the SD of five or six experiments.

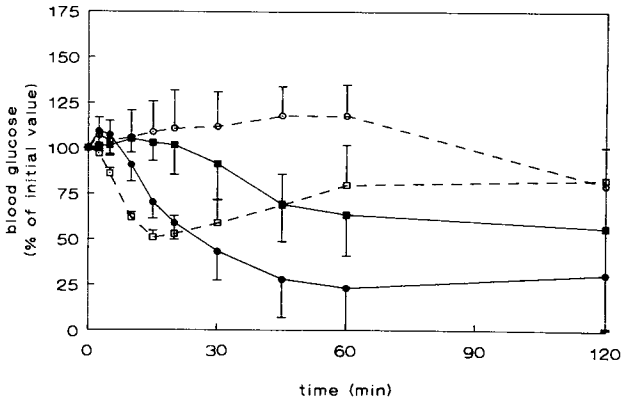

Fig. 2. Mean blood glucose concentrations after intravenous administration of 0.05 IU insulin (□), nasal administration of placebo (○), and nasal administration of 0.4 IU insulin with 5% DMβCD (●) and 5% α-CD (■). Bars represent the SD of five or six experiments.

Table I. Pharmacokinetics and Pharmacodynamics of Intravenously and Intranasally Administered Insulin to Rats[a]

Dose (μg)	Route	Additive (%, w/v)	T_{max} insulin (min)	C_{max} insulin (μU/ml)	AUC insulin (μU · ml^{-1} · min)	AUC glucose (% of initial values * 10^3)	F insulin (%)	(N)
2.5	i.v.	—	2.5	663 ± 176	5610 ± 1850	8.9 ± 1.9	100	(5)
0	i.n.	Placebo	—	—	2922 ± 777	12.7 ± 1.3	0	(5)
20	i.n.	α-CD (5%)	30	115 ± 42	8873 ± 2364**	8.8 ± 3.1*	27.7 ± 11.5	(6)
20	i.n.	β-CD (1.8%)	—	—	3553 ± 1144	12.8 ± 5.5	2.9 ± 5.3	(4)
20	i.n.	γ-CD (5%)	—	—	2074	14.9	0	(2)
20	i.n.	DMβCD (5%)	10	737 ± 164	26346 ± 7818**, ***	4.8 ± 2.3**, ***	108.9 ± 36.4	(6)
20	i.n.	HPβCD (5%)	—	—	3172	14.2	1.2	(2)

[a] All values are the mean ± SD for the number of rats given in parentheses (N). AUC represents the area under the serum/blood concentration–time curve as determined to 2 hr. F represents the absolute bioavailability.
* Significantly different from nasal placebo administration: $P < 0.05$.
** Significantly different from nasal placebo administration: $P < 0.005$.
*** Significantly different from nasal administration of insulin with α-CD: $P < 0.05$.

μg insulin, absolute bioavailabilities of 100 and 28% can be calculated for DMβCD and α-CD, respectively (Table I).

Ciliary Beat Frequency Measurements

The effects of the undiluted cyclodextrins, at concentrations as used in the nasal insulin absorption studies, on the ciliary beat frequencies of chicken embryo trachea *in vitro* are depicted in Table II. The CBF in the control experiments (LR) remained between 95 and 100% throughout the experiment. HPβCD (5%), β-CD (1.8%), and γ-CD (5%) reduced the CBF values to 65–80% of their initial frequencies within 10 min, whereafter they remained fairly constant. Both DMβCD and α-CD at concentrations of 5% induced ciliostasis within 30 to 40 min. Because DMβCD is the most potent enhancer of nasal insulin absorption, we measured the reversibility of the effect on ciliary activity. When after a 20-min exposure to 5% DMβCD the ciliated tissue was replaced in pure LR, the ciliary movement appeared to recover (Fig. 3).

DISCUSSION

DMβCD at a concentration of 5% (w/v) is a very potent enhancer of nasal insulin absorption, leading to complete bioavailability following nasal administration in rats. It has been suggested that cyclodextrins may extract lipids from the gastrointestinal mucosa, thereby leading to facilitated oral drug absorption (14). Such a mechanism might also be implicated in their enhancing effect on nasal insulin absorption. *In vitro* studies with human erythrocytes revealed that CDs can solubilize various components from the membranes of these cells, with different CDs possessing different solubilizing potencies (15). Other absorption enhancing mechanisms may also be involved. DMβCD and α-CD may enhance nasal insulin absorption by decreasing the metabolic polypeptide degradation in the nasal mucosa. Interaction of insulin molecules with DMβCD and α-CD may also inhibit the formation of insulin aggregates. It is well known that insulin can associate into dimers, tetramers, hexamers, and macromolecular aggregates, dependent on the peptide concentration and the pH of the dosage form (7). Since it is believed that only the insulin monomers can penetrate the mucosal barrier, a decreased aggregate formation may result in improved insulin absorption.

A major challenge in nasal drug delivery is to obtain reproducible absorption profiles. The use of bioadhesive microspheres for nasal administration (19,20) represents an attractive approach in reducing the interindividual variability in nasal drug absorption. The presented data on the variability in insulin absorption with DMβCD seem to be in the same order of magnitude as shown for bioadhesive microsphere preparations (19,20).

Table II. Effects of Cyclodextrins on Ciliary Beat Frequency (CBF) of Chicken Embryo Trachea *In Vitro*[a]

Additive	% (w/v)	Time (min) 10	20	30	40	50	60
Placebo		97 ± 7	101 ± 11	97 ± 6	98 ± 5	95 ± 10	98 ± 10
α-CD	5	35 ± 17	17 ± 18	7 ± 12	1 ± 3	0	0
β-CD	1.8	77 ± 11	81 ± 30	70 ± 10	71 ± 16	62 ± 20	63 ± 22
γ-CD	5	72 ± 9	66 ± 10	70 ± 13	72 ± 6	70 ± 13	77 ± 14
DMβCD	5	48 ± 11	22 ± 13	1 ± 1	0	0	0
HPβCD	5	65 ± 14	68 ± 16	77 ± 14	81 ± 25	78 ± 14	78 ± 12

[a] All data are presented as percentages of the initial values (t_0 = 100%) and are the mean ± SD of four experiments.

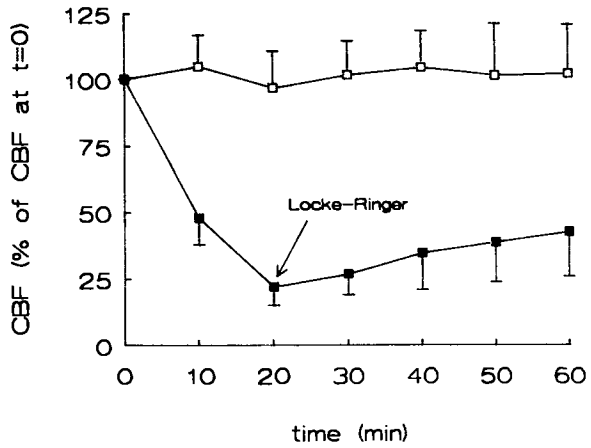

Fig. 3. Mean ciliary beat frequency of chicken trachea *in vitro:* placebo (Locke–Ringer) (□); exposure to 5% DMβCD until t = 20 min, followed by replacement with LR (■). Data are the mean ± SD of six experiments.

A prerequisite in nasal drug delivery is that drugs and additives in the dosage forms do not interfere with normal nasal functioning such as the nasal mucociliary clearance. Therefore, we have investigated the influence of the cyclodextrins, used in the present nasal insulin absorption studies, on the ciliary beat frequency of chicken embryo tracheal tissue *in vitro,* as a useful model of human nasal ciliated tissue (21). DMβCD at a final concentration of 5% (w/v) causes a moderate ciliostatic effect, but this effect is reversible. The ciliostatic potency of DMβCD is much less than found for STDHF (1%), laureth-9 (0.3%), deoxycholate (0.3%), glycocholate (1.5%), and taurocholate (1.5%) (18,22). The inhibitory effect of compounds on the ciliary movement *in vitro* is probably more pronounced than that *in vivo. In vitro,* the ciliated tissue is directly exposed to the compounds investigated, whereas *in vivo* the cilia are protected by the mucus layer. Moreover, under *in vivo* conditions the administered drugs and additives will be diluted by the nasal mucus layer and subsequently eliminated by the nasal mucociliary clearance. In view of the 100% insulin bioavailability, obtained after coadministration with 5% (w/v) DMβCD, lower concentrations of DMβCD will probably also lead to improved insulin absorption. We recently demonstrated that a final concentration of 2% DMβCD showed only minor effects on human nasal ciliary movement *in vitro* (13) and that a nasal formulation of estradiol containing 2% DMβCD, administered to oophorectomized women twice daily over a period of 6 months, was well tolerated by these patients (23). The nasal absorption of insulin in the presence of varying concentrations DMβCD in rats is presently under investigation.

In conclusion, nasal insulin absorption is remarkably promoted by the cyclodextrin derivative DMβCD. Coadministration of 5% (w/v) DMβCD results in complete insulin bioavailability and a concomitant strong hypoglycemic response in rats. Because of its high water solubility, DMβCD is largely excreted in the urine after intravenous or intramuscular administration in rabbits (14), making severe nephrotoxic effects of this compound unlikely. DMβCD has been reported to elicit hemolytic activity on human erythrocytes *in vitro* (24). Even after complete absorption of DMβCD from our nasal formulation, DMβCD blood levels are much too low to induce hemolysis *in vivo.* Although DMβCD at the concentration used in this study shows ciliostatic potency as measured with chicken embryo tracheal tissue *in vitro,* this effect appears to be reversible.

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