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# Time dependent effects of two absorption enhancers on the nasal absorption of growth hormone in rabbits

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**Abstract**

Enhancer-based drug preparations allow absorption of peptide drugs. We investigated the reversibility with time of nasal absorption of human growth hormone (hGH) induced by the absorption enhancers didecanoylphosphatidylcholine (DDPC) and α-cyclodextrin (α-CD). Rabbits were dosed intranasally with enhancer in the absence of hGH at time −3, −1, −0.5 and 0 h. At time zero the same groups of rabbits were dosed with a hGH powder devoid of the enhancers. Values for plasma hGH AUC and $C_{max}$ were estimated in order to measure the degree of absorption, and rabbits receiving hGH together with enhancers were used as positive references. With an enhancer preparation of 8% DDPC and 30% α-CD, the hGH AUC and $C_{max}$ values showed a significant time-dependent decrease after enhancer administration. This may indicate recovery of the enhancer-induced mucosal leakiness as well as clearance of the enhancers from the tissue. Similar findings were obtained with α-CD alone, whereas a preparation containing only DDPC showed no recovery of leakiness. The effect of α-CD was also investigated in vitro in rabbit nasal mucosa mounted in Ussing chambers. Incubation for 15 min with 1, 3 and 8% α-CD induced a concentration-dependent decrease of the potential difference, the short circuit current and the resistance suggesting impaired sodium transport and loosening of tight junctions. After washout of α-CD, this effect was completely reversible for the low concentrations of α-CD, but not for the 8% α-CD. These results show that α-CD alone, or in combination with DDPC, has properties as a reversible enhancer for nasal absorption of hGH in vivo.

*Keywords:* Human growth hormone; Intranasal; Absorption enhancers; Didecanoylphosphatidylcholine; α-Cyclodextrin; Rabbits

## 1. Introduction

Intranasal delivery of human growth hormone (hGH) has been shown to be a possible alternative to the traditional subcutaneous (s.c.) route of hGH administration (Illum et al., 1990). Advan-

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*Abbreviations:* DDPC, 1,2-didecanoyl-L-3-phosphatidylcholine; α-CD, α-cyclodextrin; hGH, biosynthetic human growth hormone; AUC, area under the curve; $C_{max}$, the maximal concentration; $T_{max}$, the time when the maximal concentration is achieved; s.c., subcutaneous; i.n., intranasally; i.v., intravenous; PD, potential difference; $I_{sc}$, short circuit current; R, resistance

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tages associated with drug delivery through the nasal cavity compared to s.c. injections may include a better patient compliance due to the exclusion of injection pain and easier administration (Zia et al., 1993; Gizurarson and Bechgaard, 1991). Furthermore, the normal endogenous pulsatile GH secretory pattern (Martha et al., 1989; Winer et al., 1990) may be mimicked by intranasal (i.n.) treatment due to a rapid absorption, and a correspondingly high peak amplitude. This is desirable since frequent pulsatile GH applications to rats result in a higher growth rate compared to one daily s.c. injection of the total amount of hGH (Clark et al., 1985; Pampori et al., 1991). The drawback is, however, that intranasal application of most peptide drugs results in a very low bioavailability (1–2%), due to the high molecular weight, high hydrophilicity and metabolic lability of these compounds (McMartin et al., 1987). Coadministration of enhancers can improve the bioavailability (Muranishi, 1990). A previous study with rabbits has shown that a hGH nasal preparation based on the absorption enhancers, didecanoylphosphatidylcholine (DDPC) and α-cyclodextrin (α-CD), was well absorbed (M.K. Thomsen: unpublished data). The relative bioavailability was 20%. Evaluating the applicability of CDs as nasal absorption enhancers, the duration of the absorption enhancing effect is important. A previous study (Watanabe et al., 1992) showed that the enhancer effect of α-CD with respect to nasal insulin absorption was reversible. The aim of this study was to investigate whether the enhancer efficacy was reversible using hGH and three different enhancer preparations in rabbits in vivo, i.e. DDPC + α-CD, DDPC and α-CD. Furthermore, the effect of α-CD on the electrophysiological parameters of rabbit nasal epithelium was investigated in vitro.

## 2. Materials and methods

### 2.1. Materials

hGH powder, Norditropin® solution and DDPC were from Novo Nordisk A/S, Bagsværd, Denmark. α-CD was from Takeda (Japan) and bovine serum albumin was from Sigma, St. Louis, MO, USA.

The Ussing chambers were made by Peter Korsgaard, University of Copenhagen, August Krogh Institute, Copenhagen, Denmark.

### 2.2. Dosing preparations

#### 2.2.1. Preparations for i.n. application

Twelve milligram preparations were prepared. The basic powder consisted of 0.3 mg ethocel, 0.154 mg glycine, 0.215 mg citrate, 10% α-CD and variable amounts of methocel (up to 12 mg) depending on the amount of α-CD and DDPC added as enhancer. Preparations containing 10% α-CD were considered to be inert (data not shown). The concentration of hGH in hGH containing nasal powder preparations was 3 IE per 12 mg powder, corresponding to 1.023 mg hGH per 12 mg powder as measured after lyophilization. Powders containing 8% DDPC, 8% DDPC + 30% α-CD, or 30% α-CD were also prepared.

The preparations were generally prepared by lyophilization of hGH and α-CD followed by ethanol granulation with DDPC.

#### 2.2.2. Preparations for i.v. injections

The reference hGH solution (Norditropin®) for i.v. injection was made by adding 1 ml sterile $H_2O$ to a vial containing 4 IU hGH. Dilution to the final concentration was performed by adding an adequate volume of Norditropin® buffer — containing 1% human serum albumin – to yield a final volume of 5.3 ml.

### 2.3. Experimental animals

Forty-five New Zealand white rabbits of both sexes older than 18 weeks, and weighing 2.5–3.9 kg, were delivered from Novo Nordisk A/S, Nyvanggaard, Ganløse, Denmark. Thirty five of the rabbits were used twice after a 1 week recovery period. Significant formation of neutralizing antibodies towards hGH did not occur within this week. The animals were allowed 14 days of acclimatization and had free access to water and food.

### 2.4. Study design

For the i.n. application, a nasal dosing device consisting of a 30 ml plastic syringe with a well-defined intrasyringeal air pressure was used. The i.v. control group was dosed 100 μl/kg through the marginal ear vein.

The animals were divided into 8 groups, 3 i.n. test groups and 5 control groups. In the 3 i.n. groups the enhancing effect of α-CD ($n$ = 4–5), DDPC ($n$ = 4–5) and the combination of DDPC and α-CD ($n$ = 4–5) was tested. In the control groups following experiments were performed: the absorption of hGH without enhancers ($n$ = 5), the absorption of hGH and α-CD in one powder, hGH and DDPC in one powder, hGH and α-CD plus DDPC in one powder ($n$ = 5 for each experiment) as well as hGH dosed i.v. ($n$ = 5). For each 3 i.n. test group, the animals were given hGH at t = 0 h. Enhancers, DDPC and α-CD, were given either individually or in combination at t (h) = −3, −1, −0.5 or 0. Animals, which received enhancers and hGH in separate powders at t = 0, were used as controls compared to animals, which received hGH and enhancers at different times.

### 2.5. Blood samples and radioimmunoassay

Following administration of hGH (i.n. or i.v.), 0.5 ml heparinized blood samples were obtained in ice cold tubes at 12 different time points.

The blood samples were centrifuged and the amount of immunoreactive hGH in plasma was determined by a hGH ELISA assay (Thomsen et al., 1994).

### 2.6. Statistics

The mean ± S.E.M. of the hGH plasma concentrations was calculated in each case, and following estimation of the AUCs, the relative bioavailability of i.n. hGH was determined as: $F_{rel} = AUC_{i.n.} \times D_{i.v.}/AUC_{i.v.} \times D_{i.n.}$ where $F_{rel}$ = relative bioavailability, AUC = area under the curve, and D = dose administered. Statistical significance was assessed by one-way analysis of variance (ANOVA), the level of significance being set at $P < 0.05$.

### 2.7. Tissue preparation

For the electrophysiological experiments the rabbit nasal mucosa was prepared as described by Carstens et al., 1993. The tissue was mounted between the two halves of an Ussing chamber (exposed area 0.4 $cm^2$) and equilibrated for at least 1 h in oxygenated Krebs Ringer buffer, pH 7.4 at 37°C.

### 2.8. Ussing chamber technique

The volumes in the half chambers of the Ussing chamber setup was 7 ml and 6 ml on the mucosal and serosal side, respectively. The Krebs Ringer solution in each chamber was oxygenated and circulated by a gaslift using 95% $O_2$ + 5% $CO_2$. The temperature was kept at 37°C. One percent (10 mM), 3% (31 mM) and 8% (82 mM) α-CD was added to the mucosal side and incubated for 15 min. Thereafter α-CD was washed out with Krebs Ringer buffer and the electric parameters were followed for up to 4.5 h. The PD and $I_{sc}$ across the nasal epithelium were measured by two Ag/AgCl electrodes connected on each side of the tissue surface via Ringer – 3% agar bridges.

The serosal side was electrically positive relative to the mucosal side. The electrodes were connected to a DVC-1000 Voltage/Current Clamp (World Precision Instruments). PD and $I_{sc}$ were recorded on a double channel recorder (Kipp and Zonen). Correction for fluid resistance was made. Constant voltage pulses of 1 mV with a duration of 10 s and intervals of 100 s were passed across the tissue. Changes in $I_{sc}$ were used to calculate R. PD, $I_{sc}$ and R values are expressed in mV, μA/$cm^2$ and Ohm*$cm^2$, respectively.

## 3. Results

### 3.1. Intravenous experiment

Following i.v. application of 64 μg hGH per 2.5 kg body weight to 5 rabbits, the plasma kinetics of hGH could be described as following first order elimination kinetics (data not shown). The AUC was 3824 ± 396 ng/ml*min ($n$ = 5). This mean value was used in the $F_{rel}$ calculations.

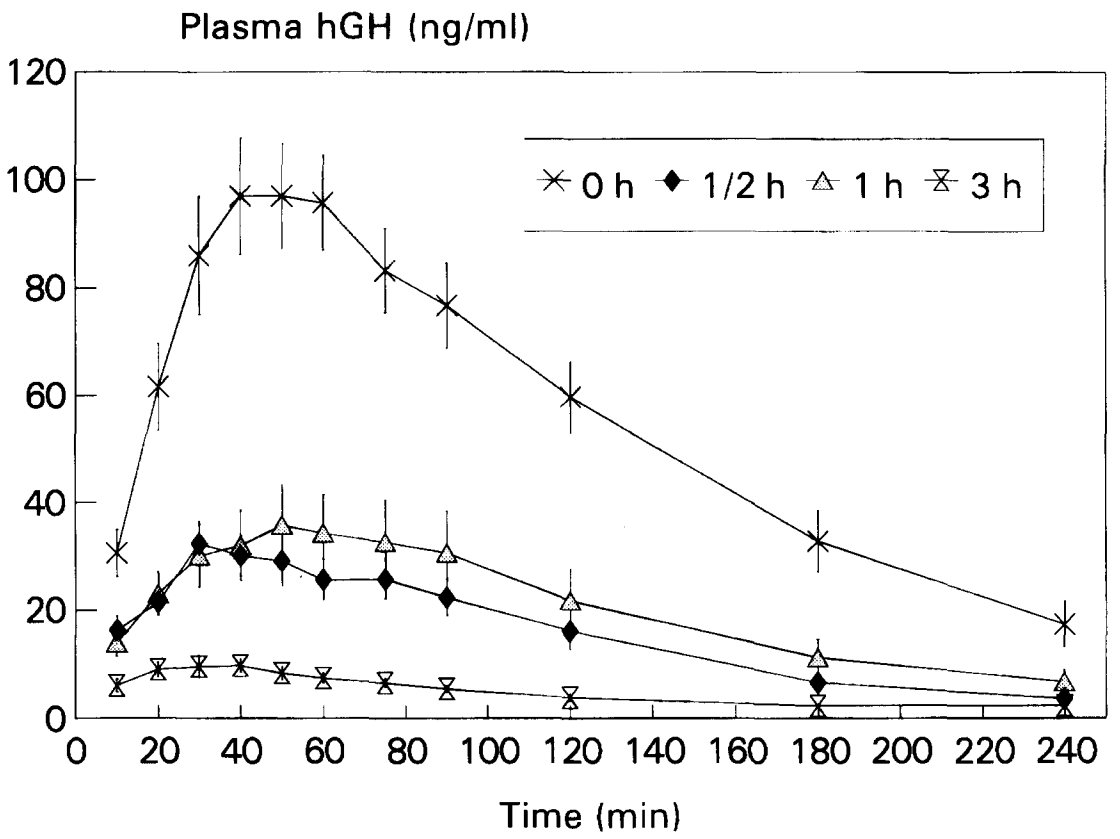


Fig. 1. Plasma concentrations of hGH after intranasal administration of hGH and α-CD in separate powders. The hGH and α-CD powders were given at the same time and with a time difference of 0.5, 1 and 3 h between the initial α-CD and subsequent hGH dosing.

### 3.2. Intranasal experiments

Administration of the basic powder including hGH resulted in a bioavailability of 8.3 ± 0.8% ($n$ = 5). This is a high absorption. Normally intranasal enhancer-free application of most peptide drugs results in a bioavailability of only 1–2% (McMartin et al., 1987).

The plasma concentrations of hGH indicated that the hGH was absorbed quickly into the systemic circulation. The highest concentration of hGH was achieved when the enhancers and hGH were dosed at the same time (Fig. 1, Fig. 2 and Fig. 3).

A bioavailability of 23.6 ± 2.4% ($n$ = 5) was obtained when hGH and α-CD were administered as one powder. However, simultaneous administration of hGH and α-CD in separate powders resulted in a $F_{rel}$ of 18.1 ± 2.0% ($n$ = 4). This lower bioavailability may be due to a possible different localization of the hGH- and α-CD powders.

The AUC obtained after administration of hGH + α-CD (13071 ± 1440 ng/ml*min, $n$ = 4) was significantly different from the AUCs obtained when hGH was administered at time intervals after the enhancer (t = 0.5 h: 3730 ± 550 ng/ml*min ($P < 0.05$) ($n$ = 5), t = 1 h: 4844 ± 1103 ng/ml*min ($P < 0.05$) ($n$ = 4), t = 3 h: 1123 ± 133 ng/ml*min ($P < 0.05$) ($n$ = 4) (Fig. 4). This suggests a time dependent effect of the enhancer and/or the repair of the mucosa.

The $C_{max}$ values follow the AUC rank order (Fig. 5), whereas there was no significant differ-

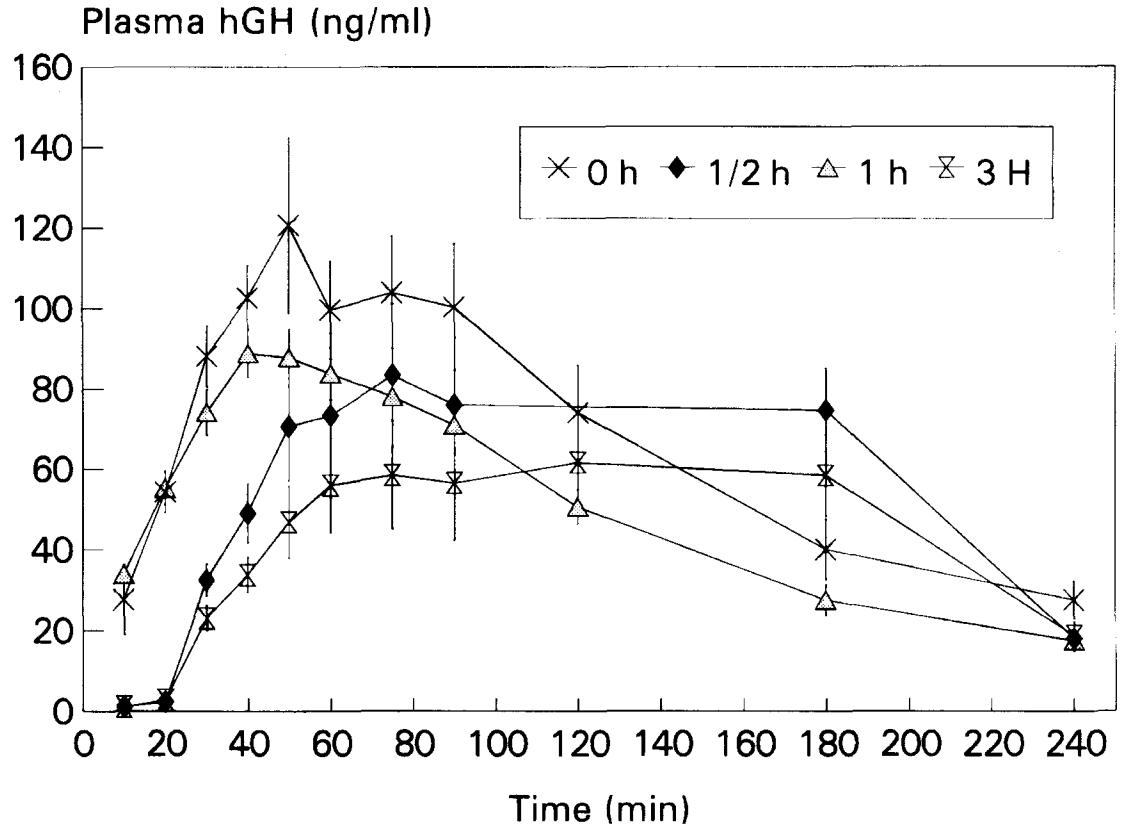


Fig. 2. Plasma concentrations of hGH after intranasal administration of hGH and DDPC in separate powders. The hGH and DDPC powders were given at the same time and with a time difference of 0.5, 1 and 3 h between the initial DDPC and subsequent hGH dosing.

ence between the different $T_{max}$ values (t = 0 h: 45 ± 3 min ($n$ = 4), t = 0.5 h: 36 ± 3 min ($n$ = 5), t = 56 ± 6 min ($n$ = 4), t = 3 h: 35 ± 3 min ($n$ = 4).

Administration of DDPC and hGH in one powder, or given at the same time in two powders resulted in $F_{rel}$ = 22.3 ± 3.4% ($n$ = 5) and 21.5 ± 2.8% ($n$ = 5), respectively. Concerning the AUC (Fig. 4), $C_{max}$ (Fig. 5) and $T_{max}$ (t = 0 h: 71 ± 9 min ($n$ = 5), t = 0.5 h: 75 ± 8 min ($n$ = 5), t = 1 h: 51 ± 8 min ($n$ = 4), t = 3 h: 69 ± 10 min ($n$ = 5)) no significant difference between the different time groups was found. This implies that no significant repair of the mucosa or reversibility of the toxicity occurred.

Simultaneous administration of DDPC + α-CD and hGH in two powders resulted in a bioavailability ($F_{rel}$) of 14.3 ± 1.6% ($n$ = 4), whereas dosing of one powder containing both enhancers and hGH resulted in $F_{rel}$ = 31.9 ± 4.2% ($n$ = 5).

In the estimated AUC values (Fig. 4), there was a significant difference between application of hormone and enhancers at time zero (10362 ± 1156 ng/ml*min ($n$ = 4)) compared to an administration of the enhancers into the nasal cavity prior to administration of the hormone (t = 0.5 h: 5680 ± 711 ng/ml*min ($P$ < 0.05) ($n$ = 5), t = 1 h: 4694 ± 643 ng/ml*min ($P$ < 0.05) ($n$ = 5), t = 3 h: 1590 ± 373 ng/ml*min ($P$ < 0.05) ($n$ = 4)).

The $C_{max}$ values follow the AUC pattern (Fig. 5) and consistent with the other groups, the $T_{max}$ values were not significant different (t = 0 h: 61

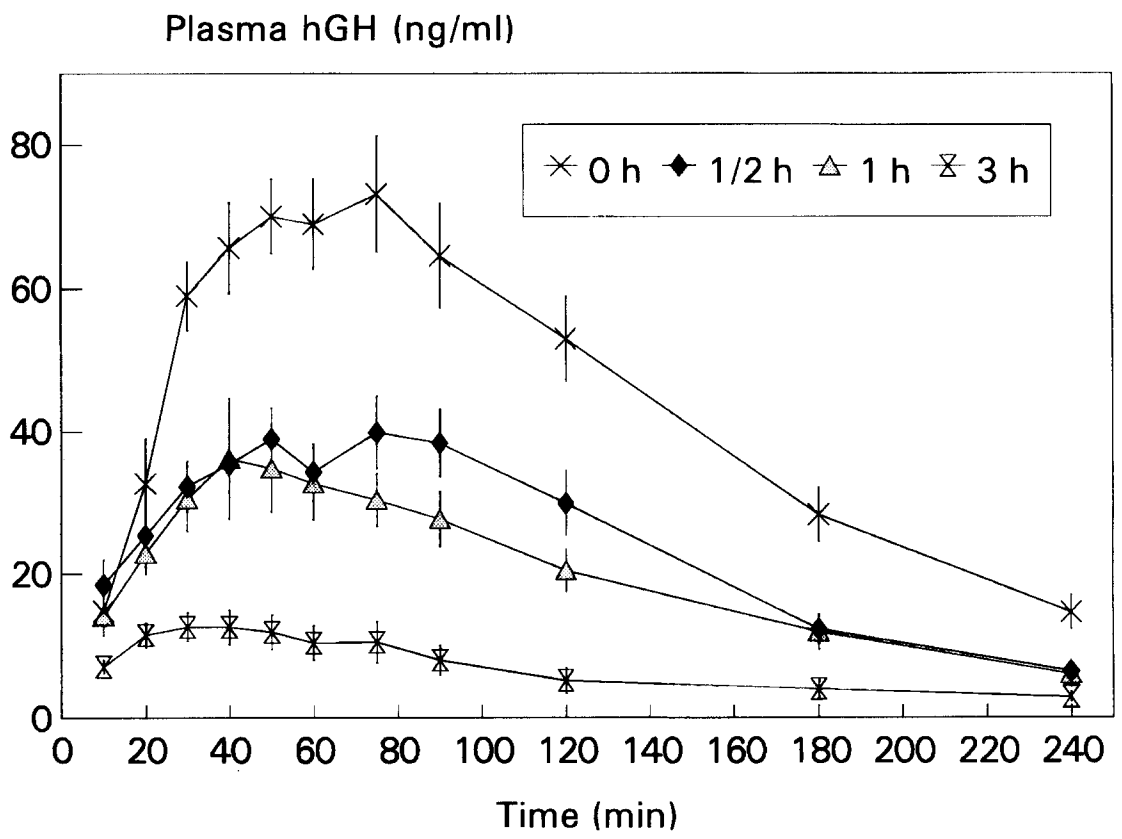


Fig. 3. Plasma concentrations of hGH after intranasal administration of hGH and α-CD plus DDPC in separate powders. The hGH and enhancer powders were given at the same time and with a time difference of 0.5, 1 and 3 h between the initial α-CD plus DDPC and subsequent hGH dosing.

± 8 min ($n$ = 4), t = 0.5 h: 64 ± 5 min ($n$ = 5), t = 1 h: 51 ± 8 min ($n$ = 5), t = 3 h: 38 ± 5 min ($n$ = 4)).

### 3.3. Electrophysiological study

After at least 1 h equilibration in Ussing chamber, the PD was 7.5 mV ± 0.8 mV ($n$ = 3) and the $I_{sc}$ was 50.0 $\mu A/cm^2$ ± 15.1 $\mu A/cm^2$ (n = 3). The resistance was calculated to be 151.0 ± 33.1 ohm*$cm^2$ ($n$ = 3) (all values represent means ± S.E.M.).

The effects of 1, 3 and 8% α-CD on the nasal mucosa were investigated for up to 4.5 h after addition of the enhancer. Fig. 6a, Fig. 6b and Fig. 6c is from one representative experiment and shows the changes in the PD, $I_{sc}$ and R, respectively, induced by the different α-CD concentrations. One percent α-CD was applied on the mucosal side at time zero and incubation proceeded for 15 min. The electrophysiological parameters were then followed until recovery was achieved. At this time, a new preparation of 3% α-CD was applied for 15 min as shown in Fig. 6a, Fig. 6b and Fig. 6c. In the same experiment 8% α-CD was later applied. For all parameters, time courses of the three electrical parameters showed that 1% was close to the no effect level. Three percent and 8% α-CD caused an initial reduction with subsequent complete or partial recovery of all parameters, the effect on the PD being most pronounced (Fig. 6a, Fig. 6b, Fig. 6c and Fig. 7a, Fig. 7b and Fig. 7c).

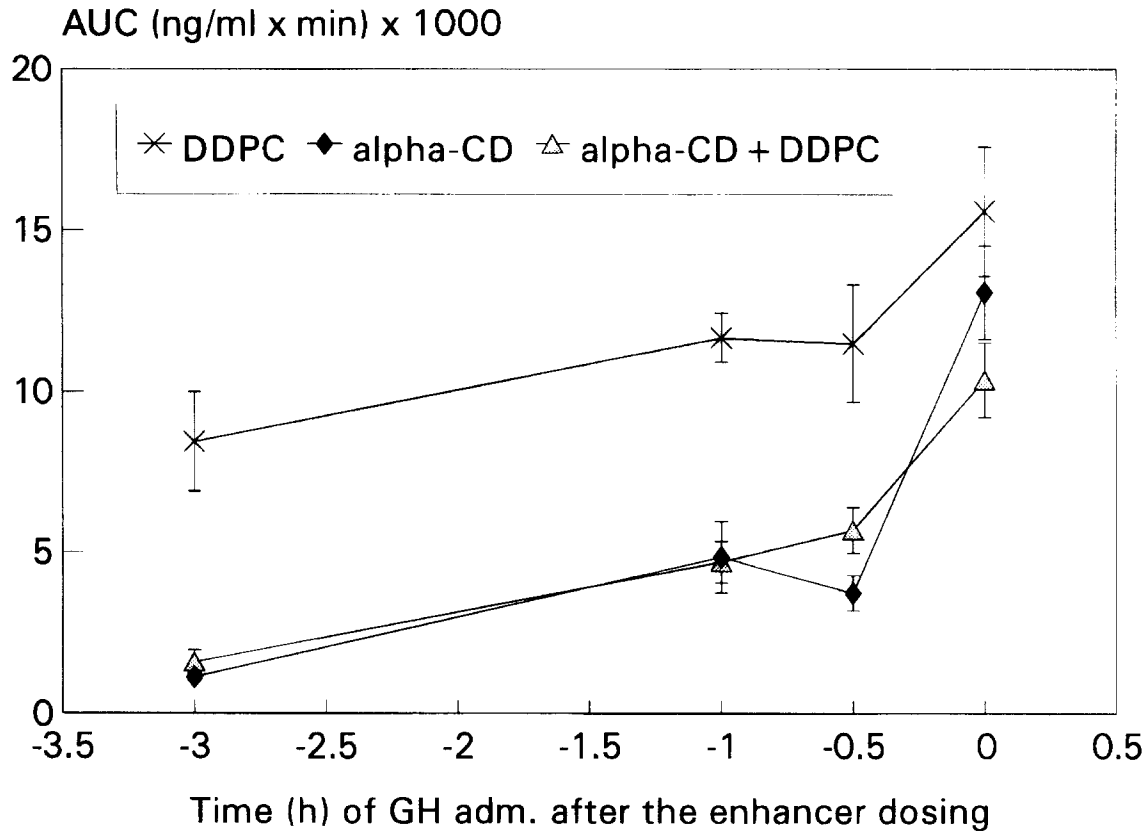


Fig. 4. Area under the curve (AUC) of hGH after administration of α-CD, DDPC and a combination of α-CD and DDPC. hGH was given at t = 0 and the enhancers were given at t = −3, −1, −0.5 or 0. Mean values ± S.E.M. are shown ($n$ = 4–5). Points represent groups of different rabbits.

For the three different electrical properties, a concentration dependent temporary reduction in all values was seen (Fig. 7a, Fig. 7b and Fig. 7c), with the longest time to recovery being observed for a concentration of 8% α-CD.

## 4. Discussion

The present study showed a time dependency of the enhanced trans-mucosal passage (i.e. the mucosal leakiness) induced by 30 wt % α-CD, or a combination of 30 wt % α-CD + 8 wt % DDPC, in rabbits in vivo. When compared to intravenously administered hGH, the relative bioavailability of hGH with these two preparations (hGH and enhancers in the same powder) was 23.6 ± 2.4% and 31.9 ± 4.2%, respectively.

Results obtained with a nasal human insulin preparation containing 2% DDPC given in the meal-relevant period showed a bioavailability (compared to s.c. administered insulin) of 23.9% in humans (Drejer et al., 1992). Merkus et al. and Schipper et al. found a nasal insulin bioavailability of approx. 30% in rats (by co-administration of 5% α-CD) (Merkus et al., 1991; Schipper et al., 1992), whereas Watanabe et al. obtained a bioavailability of 5% dosing 15% α-CD in rabbits (Watanabe et al., 1992).

The relatively low AUC and $C_{max}$ values obtained in the experiments using simultaneous administration of hGH and enhancer in two individual powders, compared to using only one powder with both substances included, indicates that either the enhancer needs to interact directly

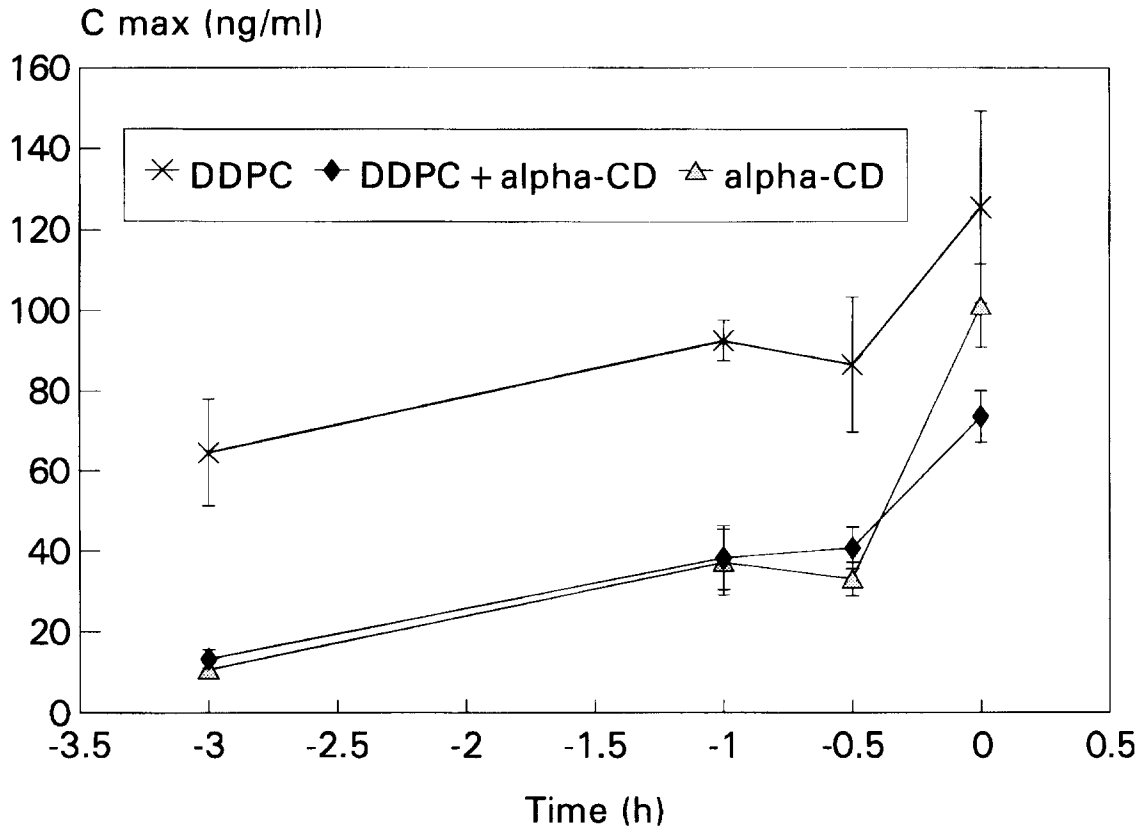


Fig. 5. The maximal concentration ($C_{max}$) of hGH after administration of α-CD, DDPC and a combination of α-CD and DDPC. Growth hormone was given at t = 0 and the enhancers were given at t = −3, −1, −0.5 or 0. Mean values ± S.E.M. are shown ($n$ = 4–5). Points represent groups of different rabbits.

with hGH present in the formulation, or exact co-localization of the two powder doses on the mucosa did not occur.

The AUC and $C_{max}$ obtained by simultaneous co-administration of hGH and the enhancer formulations containing α-CD, and α-CD + DDPC in combination, were significantly different as compared to the values obtained by delayed administration of hormone (Figs. 4 and 5). The absorption of a dose of hGH in enhancer-free formulation, applied 0.5 h after dosing of the two test preparations, resulted in AUC and $C_{max}$ values that had decreased by approx. one half. Three hours after enhancer dosing, the absorption of administered hGH was very low (Figs. 4 and 5). This suggests reversibility of the enhancer effect which may be due to clearance of the enhancers as well as a time-dependent repair of the mucosal absorption barrier. Similar to these findings, a 12 h pre-administration of dimethyl-β-CD results in significantly lower values of $C_{max}$ and AUC than those obtained by the simultaneous administration of insulin and dimethyl-β-CD to rabbits (Watanabe et al., 1992).

The recovery of barrier function in vivo is in agreement with the present electrophysiological study of the influence of α-CD on the nasal epithelium in vitro. These results demonstrated a concentration dependent decrease in the electrophysiologic parameters following a 15 min incubation with α-CD (Fig. 7a, Fig. 7b and Fig. 7c). The three concentrations (1% (10 mM), 3% (31 mM), 8% (82 mM)) of α-CD exhibited complete or partial reversibility of the PD, $I_{sc}$ and R (Fig. 6a, Fig. 6b and Fig. 6c).

The reduction of PD may be caused by blocking of the $Na^+$ channels in the mucosa membrane or by opening of tight junctions. The latter inter-

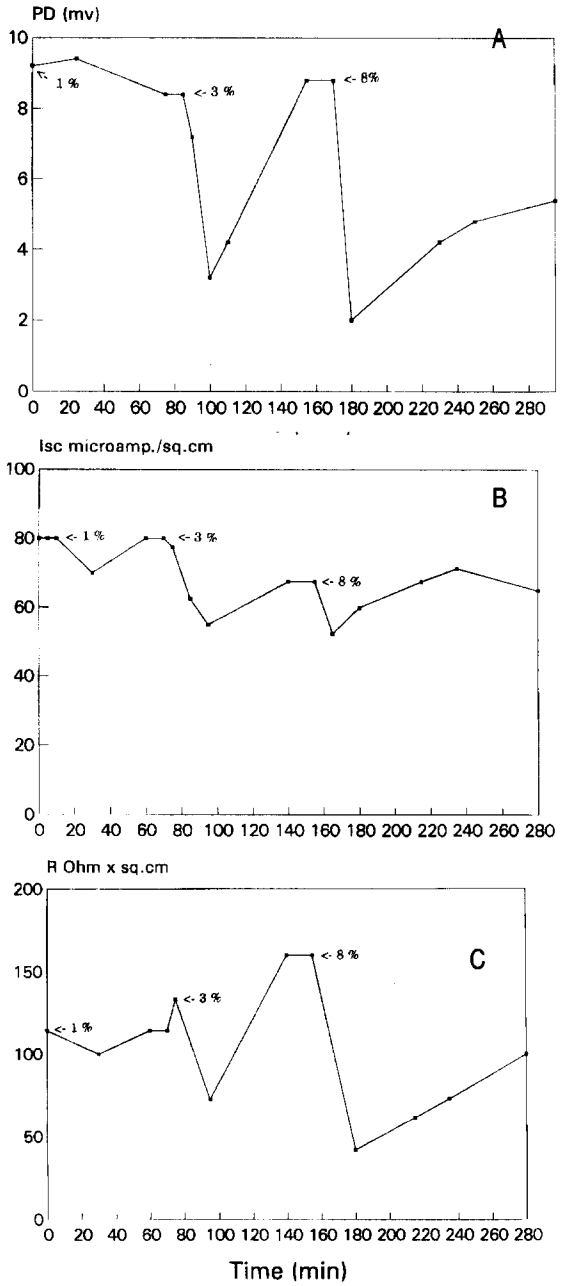


Fig. 6. Changes in potential difference (PD), short circuit current ($I_{sc}$) and resistance (R) with time after 15 min application of 1, 3 and 8% α-CD to the mucosal side of the nasal epithelium mounted in an Ussing chamber.

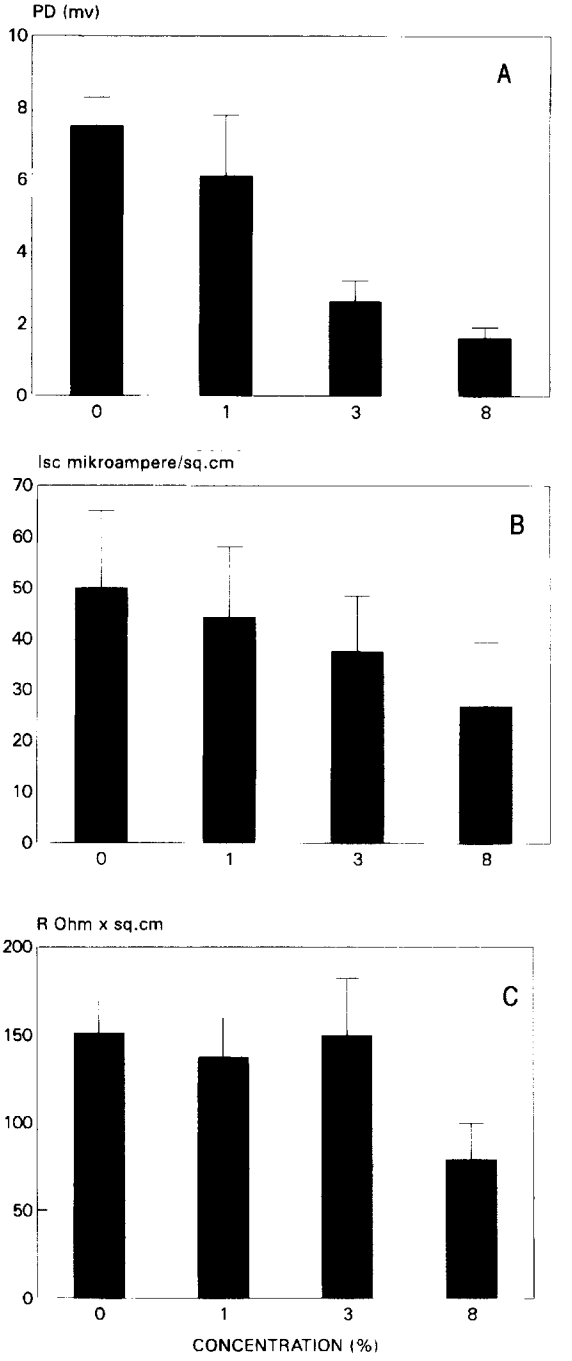


Fig. 7. Concentration-dependent reduction of the electrical properties by 15 min application of α-CD to the mucosal side of the nasal tissue mounted in an Ussing chamber. Mean values ± S.E.M. of the maximal reduction in the measured parameters are shown ($n$ = 3).

pretation is supported by the R measurements. Reduction of $I_{sc}$ indicates a reduction of the active ion transport, i.e. reduction of the $Na^+$ permeability of the mucosa membrane and the activity of the $Na^+$, $K^+$-pump in the serosa membrane. α-CD has been reported to extract membrane components (Ohtani et al., 1989; Shao et al., 1992) and this effect may be causally related to the deterioration of the electrophysiological properties in the present in vitro system. Thus, the present electrophysiologic data may suggest increased permeability via the paracellular route. Nakanishi et al. (1992), however, suggested that 10 mM β-CD, after pre-treatment with a mucolytic agent, mainly enhanced the permeability of the transcellular pathway. Lowering of the cholesterol content of the membrane by β-CD, was suggested in this study to cause an accentuation of the transcellular route (Nakanishi et al., 1992).

In contrast to the above mentioned in vivo results, no significant difference in AUC, $C_{max}$ and $T_{max}$ was observed in the groups receiving DDPC and hGH at same time or temporarily dissociated (Figs. 4 and 5). This suggests an irreversible and perhaps toxic effect of DDPC. This is in agreement with previous electrophysiological studies (Bechgaard et al., 1993; Vermehren et al., 1993) of the effect of DDPC on the nasal mucosa in vitro and should furthermore be considered when designing DDPC-containing nasal preparations for clinical use. Two percent DDPC gave a less complete recovery of the electrophysiological parameters than did 8% α-CD. The present in vivo experiment – using powder with 8% DDPC – showed also a more prolonged leakiness as compared to the preparation with 30% α-CD. Electron microscopic studies of rabbit nasal epithelium have shown that DDPC may promote destruction of cells and suggested a transcellular hGH transport through these damaged cells (Agerholm et al., 1994).

When DDPC is dosed in combination with α-CD, the CD may protect the membrane against toxicity induced by DDPC (De Ponti et al., 1992; Irie et al., 1992; Gill et al., 1994a; Gill et al., 1994b). α-CD consists of six glucose residues arranged in a rigid conical conformation, with a hollow interior. It is capable of forming inclusion complexes with polar hydrophobic compounds. It is known that α-CD forms inclusion complexes with phosphatidylcholines in water (Miyajima et al., 1985). Depositing the powder on the mucosa may initiate complex formation between DDPC and α-CD, thus reducing the accessible enhancer concentration. Apart from the complexing between DDPC and CD, some interactions between the cyclodextrins and the components of the epithelium may occur, thereby protecting against the DDPC effect as well (De Ponti et al., 1992).

In contrast to α-CD, we hypothesise that DDPC is incorporated into the membrane, hereby prolonging the enhancing effect. A DDPC-induced opening of tight junctions (Vermehren et al., 1993) may result from incorporation of the phospholipid into the outer leaflet of the membrane, thereby expanding the outer membrane surface. This may induce changes in the cytoskeleton (Roelofsen et al., 1989) and possibly the tight junctions.

The absorption enhancing effect of α-CD may be caused by increased nasal permeability (Nakanishi et al., 1992; Irie et al., 1992). One cause for increased permeability could be opening of tight junctions possibly resulting from interference with membrane components. CD's are known to interact with the gastrointestinal mucosa by extraction of membrane components (Nakanishi et al., 1992) and solubilizing components from the erythrocyte membrane (Ohtani et al., 1989). Release of membrane components depends on the cavity size of the CD. α-CD is shown to selectively release phospholipids from erythrocyte membranes (Ohtani et al., 1989) and rat small intestine (Nakanishi et al., 1992). Similar mechanisms are probably relevant to the effects of CDs on the permeability of the nasal epithelium. Alternative mechanisms may include reduction of the ciliary beat frequency (Merkus et al., 1991), resulting in increased contact time between hGH and mucosa and suppression of the enzymatic degradation of peptides by α-CD as shown in rat nasal homogenates (Merkus et al., 1991; Irie et al., 1992).

The present results showed that enhancer preparations of α-CD and α-CD + DDPC both resulted in an increased nasal absorption of hGH

in vivo, an effect that was reversible with time. The enhancing effect of DDPC alone was not reversible within 3 h. In vitro studies of electrophysiological parameters of α-CD on rabbit nasal mucosa also showed that the leakiness of the tissue was reversible with time. In conclusion, the study documents that assessment of the reversibility of action is different between absorption enhancers such as α-CD and DDPC, and these differences may have safety implications when considering long term clinical use of the enhancers.

## Acknowledgements

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## References

Agerholm, C., Bastholm, L., Johansen, P.B., Nielsen, M. and Elling, F., Epithelial transport and bioavailability of intranasally administered human growth hormone formulated with the absorption enhancers didecanoyl-L-α-phosphatidylcholine and α-cyclodextrin in rabbits. *J. Pharm. Sci.*, 83 (1994) 1706–1711.

Bechgaard, E., Jørgensen, L., Larsen, R., Gizurarson, S., Carstensen, J. and Hvass, Aa., Insulin and didecanoyl-L-α-phosphatidylcholine in vitro study of the transport through rabbit nasal mucosal tissue. *Int. J. Pharmaceutics*, 89 (1993) 147–153.

Carstens, S., Danielsen, G., Guldhammer, B. and Frederiksen, O., Transport of insulin across rabbit nasal mucosa in vitro induced by didecanoyl-L-α-phosphatidylcholine. *Diabetes*, 42 (1993) 1032–1040.

Clark, R.G., Jansson, J.O., Isaksson, O. and Robinson, C.A.F., Intravenous growth hormone: growth responses to patterned infusions in hypophysectomized rats. *J. Endocrinol.*, 104 (1985) 53–61.

De Ponti, R., Martini, A., Crivellente, M., Artico, R., Rialdi, G., Rivella, A., Fisher, A.N., Gill, I., Farray, N.F. and Illum, L., Lowering of toxicity using cyclodextrins in combination with nasal enhancers. In-vitro and in-vivo studies. In *Proceedings of The Sixth International Cyclodextrin Symposium book of abstracts*. Chicago, April 21–24, 1992. Abstract L56.

Drejer, K., Vaag, A., Bech, K., Hansen, P., Sørensen, A.R. and Mygind, N., Intranasal administration of insulin with phospholipid as absorption enhancer: pharmacokinetics in normal subjects. *Diab. Med.*, 9 (1992) 335–340.

Gill, J., Illum, L., Farraj, N. and De Ponti, R., Cyclodextrins as protection agents against enhancer damage in nasal delivery systems. I. Assessment of effect by measurement of erythrocyte haemolysis. *Eur. J. Pharm. Sci.*, 1 (1994a) 229–236.

Gill, J., Fisher, A.N., Hinchcliffe, M., Whetstone, J., Farraj, N., De Ponti, R. and Illum, L., Cyclodextrins as protection agents against enhancer damage in nasal delivery systems. II. Effects on in vivo absorption of insulin and histopathology of nasal membrane. *Eur. J. Pharm. Sci.*, 1 (1994b) 237–248.

Gizurarson, S. and Bechgaard, E., Intranasal administration of insulin to humans. *Diab. Res. Clin. Prac.*, 12 (1991) 71–84.

Illum, L., Farraj, N.F., Davis, S.S., Johansen, B.R. and O'Hagan, D.T., Investigation of the nasal absorption of biosynthetic human growth hormone in sheep – use of a bioadhesive microsphere delivery system. *Int. J. Pharm.*, 63 (1990) 207–211.

Irie, T., Arima, H., Abe, K., Uekama, K., Matsubara, K. and Kuriki, T., Possible mechanisms of cyclodextrin-enhanced nasal absorption of peptide and protein drugs. In *Proceedings of The sixth International Cyclodextrin Symposium book of abstracts*. Chicago, April 21–24, 1992. Abstract L54.

Martha, P.M., Rogol, A.D., Veldhuis, J.D., Kerrigan, J.R., Goodman, D.W. and Blizzard, R.M., Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J. Clin. Endocrinol. Metabol.*, 69 (1989) 563–570.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Merkus, F.W.H.M., Verhoef, J.C., Romeijn, S.G. and Schipper, N.G.M., Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats. *Pharm. Res.*, 8 (1991) 588–592.

Miyajima, K., Tomita, K. and Nakagaki, M., Complex formation between di- and monophosphatidylcholines and cyclodextrins in water. *Chem. Pharm. Bull.*, 33 (1985) 2587–2590.

Muranishi, S., Absorption enhancers. *CRC. Crit. Rev. Ther. Drug Car. Syst.*, 7 (1990) 1–34.

Nakanishi, K., Tanekazu, N., Masada, M. and Miyajima, K., Effect of cyclodextrins on biological membrane. II. Mechanism of enhancement on the intestinal absorption of non-absorbable drug by cyclodextrins. *Chem. Pharm. Bull.*, 40 (1992) 1252–1256.

Ohtani, Y., Irie, T., Uekama, K., Fukunaga, K. and Pitha, J., Differential effects of α-, β- and γ-cyclodextrins on human erythrocytes. *Eur. J. Biochem.*, 186 (1989) 17–22.

Pampori, N.A., Agrawal, A.K. and Shapiro, B.H., Renaturalizing the sexually dimorphic profiles of circulating growth hormone in hypophysectomized rats. *Acta Endocrinol.*, 124 (1991) 283–289.

Roelofsen, B., Kuypers, F.S., Op den Kamp, J.A.F. and van Deenen, L.L.M., Influence of phosphatidylcholine molecular species composition on stability of the erythrocyte membrane. *Biochem. Soc. Trans.*, 17 (1989) 284–286.

Schipper, N.G.M., Verhoef, J., Romeijin, S.G. and Merkus, F.W.H.M., Absorption enhancers in nasal insulin delivery and their influence on nasal ciliary functioning. *J. Controlled Release*, 21 (1992) 173–186.

Shao, Z., Krishnamoorthy, R. and Mitra, A.K., Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluations. *Pharm. Res.*, 9 (1992) 1157–1163.

Thomsen, M.K., Hansen, B.S., Nilsson, P., Nowak, J., Johansen, P.B., Thomsen, P.D. and Christiansen, J., Pharmacological characterization of a biosynthetic trisulfide-containing hydrophobic derivative of human growth hormone: comparison with standard 22 K growth hormone. *Pharmacol. Toxicol.*, 74 (1994) 351–358.

Vermehren, C., Hansen, H.S. and Thomsen, M.K., Metabolism and effects of a phospholipid absorption enhancer in rabbit nasal epithelium in vitro. In Cevc G. and Paltauf F. (Eds.), *Proceedings of the sixth International Colloquium on Phospholipids, Hamburg, Oct.* 1993, AOCS, Champaign, pp. 82–102 (in press).

Watanabe, Y., Matsumoto, Y., Kawamoto, K., Yazawa, S. and Matsumoto, M., Enhancing effect of cyclodextrins on nasal absorption of insulin and its duration in rabbits. *Chem. Pharm. Bull.*, 40 (1992) 3100–3104.

Winer, L.M., Shaw, M.A. and Baumann, G., Basal plasma growth hormone levels in man: new evidence for rhytmicity of growth hormone secretion. *J. Clin. Endocrinol. Metabol.*, 70 (1990) 1678–1686.

Zia, H., Dondeti, P. and Needham, T.E. Intranasal drug delivery. *Clin. Res. Reg. Aff.*, 10 (1993) 99–135.