Br. J. Pharmacol. (1993), **110**, 1335–1340

Nasal administration of an ACTH(4–9) peptide analogue with dimethyl-β-cyclodextrin as an absorption enhancer: pharmacokinetics and dynamics

Nicolaas G.M. Schipper, [1]J. Coos Verhoef, Larissa M. De Lannoy, Stefan G. Romeijn, *Jan H. Brakkee, *Victor M. Wiegant, *Willem Hendrik Gispen & Frans W.H.M. Merkus

Leiden/Amsterdam Center for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands and *Department of Medical Pharmacology, Rudolf Magnus Institute, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands

1 The systemic absorption and the neurotrophic effect of the metabolically stabilized ACTH (4–9) analogue, Org2766, were investigated following intranasal (i.n.) administration.

2 Without additives the nasal bioavailability of the peptide was in the order of 15 and 10% in rats and rabbits, respectively. The absorption could be improved by addition of a variety of absorption enhancers to the nasal preparation. The β-cyclodextrin derivative, dimethyl-β-cyclodextrin (DMβCD) at a concentration of 5% (w/v) improved the absorption in rats about 5 fold from 13 ± 4% (mean ± s.d.) for administration of the peptide alone to 65 ± 21%, and in rabbits 1 to 2 fold, from 10 ± 6% to 17 ± 8%.

3 The increased permeability of the rat nasal mucosa for Org2766 caused by DMβCD in rats reversed substantially within 1 h. However, the nasal absorption had not yet completely returned to the level without enhancer.

4 S.c. administered Org2766 accelerated the functional recovery from peripheral nerve damage in rats. However, the peptide did not facilitate nerve repair following i.n. administration with DMβCD, in spite of the fact that Org2766 was well absorbed. I.v. injection of Org2766 was also ineffective.

Keywords: Nasal delivery; dimethyl-β-cyclodextrin (DMβCD); ACTH(4–9) analogue (Org2766); absorption enhancer; peripheral nerve regeneration; peptide

Introduction

The metabolically stabilized ACTH(4–9) hexapeptide analogue, Org2766, stimulates the outgrowth of developing or damaged neurones (Strand *et al.*, 1991). In animal models that measure functional, electrophysiological and histological parameters, Org2766 has been shown to facilitate nerve repair following CNS as well as peripheral nerve lesions (Wolterink *et al.*, 1990; Spruyt *et al.*, 1990; Van der Zee *et al.*, 1991). Moreover, the peptide is clinically effective in cisplatin-induced neuropathies (Gerritsen Van Der Hoop *et al.*, 1990).

As in many peptide and protein drug therapies, the ACTH(4–9) analogue is administered parenterally. Parenteral injection therapy, however, is associated with social and physical discomfort, and may lead to poor patient compliance. Evidently, there is a need for alternative delivery routes. The absorption of orally administered peptides is poor mostly because of gastro-intestinal breakdown and to large first-pass elimination in the liver. Following peroral delivery, Org2766 did not improve the recovery from peripheral nerve damage in rats (Dekker *et al.*, 1987; Van der Zee *et al.*, 1988). Therefore, nasal administration may be an attractive alternative. The nasal route avoids first pass metabolism, is easily accessible and particularly suitable for self-medication. However, the bioavailability of nasally administered peptides and proteins is usually low because of poor permeability of the nasal epithelium for high molecular weight and hydrophilic compounds, enzymatic degradation in the nasal mucosa and the rapid mucociliary clearance resulting in a short residence time in the nasal cavity (Chien *et al.*, 1989). One approach to increase the absorption efficiency is the use of absorption enhancers in the nasal dosage form (Lee *et al.*, 1991).

It has recently been demonstrated that cyclodextrins and in particular α-cyclodextrin and the derivative dimethyl-β-cyclodextrin (DMβCD) are very potent enhancers of nasal insulin absorption in rats (Merkus *et al.*, 1991; Irie *et al.*, 1992).

In the present paper the nasal absorption of Org2766 was studied in rats and rabbits. The influence of DMβCD on the absorption was compared with the effect of other absorption enhancers. Moreover, the neurotrophic effect of the nasally administered ACTH(4–9) analogue/DMβCD dosage form on functional recovery from peripheral nerve damage in rats was studied in relation to the effect after s.c. and i.v. injections.

Methods

Animals

Male Wistar rats from an inbred strain with a body weight of approx. 200 g were used in the nasal absorption experiments. In the functional recovery studies the male Wistar rats weighed approx. 130–150 g at the start and 260–280 g at the end of these experiments. The animals were housed in Makrolan cages (5 rats/cage) and maintained at 12:12 h light: dark cycle. They were fed a commercial diet and had water *ad libitum*. The rats were randomized over the experimental groups prior to crush-lesioning of the sciatic nerve.

The rabbits used in absorption experiments were female New Zealand Whites with a body weight of 3.7–4.0 kg. They were obtained from Iffa Credo Broekman (Someren, The Netherlands), and housed in stainless steel cages. The animals were fed a commercial laboratory animal diet and had water *ad libitum*.

Preparation of peptide solutions

The ACTH(4–9) analogue was dissolved in a phosphate-buffered saline solution (pH = 7.4) (PBS), just prior to the

[1] Author for correspondence.

experiments. For intranasal (i.n.) application the absorption enhancing compounds were subsequently added to the peptide solutions. For i.v. s.c. and i.n. control administration, peptide solutions without enhancers were used. Blank solutions without peptide were 5% (w/v) DMβCD in PBS.

Absorption experiments

Male Wistar rats were anaesthetized with Hypnorm (1 ml kg^{-1} s.c.). Anaesthesia was maintained with additional injections of 0.5 ml kg^{-1} Hypnorm, usually every 45 min. Experimental animal procedures were as described previously (Schipper *et al.*, 1992b). Nasal solutions in a volume of 20 μl were instilled unilaterally through one of the nares with PVC-tubing connected to a microliter syringe. For s.c. administration a microlitre syringe was used to give a volume of 200 μl under the neck skin. Intravenous bolus injections of 20 μl were given through a cannulated femoral vein; directly afterwards the cannula was flushed with 200 μl PBS.

The rabbits were sedated with a s.c. injection of 0.5 ml kg^{-1} Hypnorm in the back to prevent sneezing. Nasal peptide solutions were instilled through one of the nares. PVC-tubing connected to a microlitre syringe was inserted 1.0 cm into a nostril and 50 μl drug solution was instilled. Intravenous peptide solutions were given in the right ear vein. Nasal and i.v. Org2766 formulations were given in a random order to each of the rabbits. A wash out period of 3 to 7 days was allowed between subsequent administrations.

Blood samples of approx. 300 μl were taken at regular time intervals. The samples were immediately put on ice, and were allowed to clot for 2 h. They were centrifuged at 4°C to obtain serum. The samples were stored at − 20°C until analysis.

Measurement of Org2766 serum concentrations

A radioimmunoassay procedure was used to determine the serum concentrations of the ACTH(4–9) peptide analogue (Gispen & Wiegant, 1990). A rabbit antiserum raised against bovine thyroglobulin-conjugated Org2766 was used at a final dilution of 1:4000. Aliquots of serum samples (10–50 μl) adjusted to 100 μl with buffer (PBS with 0.1% bovine serum albumin and 0.2% Tween-80), and 100 μl standard Org2766 in buffer were added to 50 μl antiserum. They were preincubated at 4°C for 16 h, followed by incubation for 24 h at 4°C with [^{125}I]-Org2766 (50 μl; 10,000 c.p.m.). The [^{125}I]-Org 2766 was prepared with a Chloramine-T labelling method. Antibody-bound and free Org2766 were separated by fractional precipitation with 20% (w/v) polyethylene glycol in PBS. Serum samples were assayed in duplicate. The sensitivity of the assay (10% displacement of tracer) was 1 pg/tube. The cross reactivity of the antiserum was (expressed on mass basis): [Met(O_2)^4,D-Lys^8,Phe^9]ACTH(4–9) (Org2766), 100%; [D-Lys^8,Phe^9]ACTH(4–9), 0.85%; [Acetyl-Met(O_2)^4,D-Lys^8, Phe^9]ACTH(4–9), 1.6%; [D-Lys^8, Phe^9] ACTH(5–9), 50%; [D-Lys^8,Phe^9]ACTH(6–9), 62%; [D-Lys^8,Phe^9]ACTH (7–9), 6.4%; ACTH(4–10), 0.001%.

Analysis of absorption data

The areas under the individual serum concentration-time curves (AUC) were calculated with the linear trapezoidal rule up to 3 h post administration. Nasal bioavailabilities were calculated according to the formula: $AUC_{i.n.}/AUC_{i.v.} \times D_{i.v.}/D_{i.n.} \times 100\%$, in which D is the administered dose. The raw rat AUC, time to peak concentration and peak concentration data were evaluated for statistical significant differences by a one way analysis of variance. The rabbit data were evaluated by a Student's *t* test for paired results. Differences were assigned to be significant for values of $P < 0.05$. All data are presented as the mean ± s.d.

Functional recovery from peripheral nerve damage

Rats were anaesthetized with Hypnorm (0.8 ml kg^{-1}). A crush-lesion was made in the right sciatic nerve as described in detail by De Koning *et al.* (1986). The nerve was crushed for 30 s with a haemostatic forceps. Nasal, i.v. and s.c. administrations of Org2766 were given immediately after the sciatic nerve was crushed, and subsequently every 48 h for 24 days. The rats were anaesthetized with 0.4 ml kg^{-1} Hypnorm just prior to nasal instillation of the drug solutions, and kept lying on their backs during and until 30 min after administration.

The return of sensorimotor function was measured by the foot reflex withdrawal test described previously by De Koning *et al.* (1986). A small electric current was applied to the sole of the rats foot. A positive response was scored when rats withdrew their feet from the electric stimulus instantaneously. A range of six brief current pulses from 0.1 to 0.6 mA was applied to determine the minimal current required to evoke a response. Rats were considered fully recovered when they retracted their paws at 0.1 mA (100% recovery). Failure to retract at 0.6 mA was taken as 0% recovery. The rats were tested on days 3 and 10 after crush-lesioning to check the effectiveness of the crush-lesion in the sciatic nerve. From days 14 to 24 the functional recovery was determined daily. On days that the functional recovery test coincided with peptide delivery, the recovery test was performed prior to peptide administration. The functional recovery experiments were performed double-blind. The data were expressed as the mean percentage recovery per day (± s.e. mean). Group differences were analysed by a Mann-Whitney test using the data before transformation to percentage recovery.

Chemicals

The ACTH(4–9) analogue, Org2766 (H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH) was a gift from Organon (Oss, The Netherlands). Dimethyl-β-cyclodextrin (DMβCD) and α-cyclodextrin (α-CD) were from Avebe (Foxhol, The Netherlands). Sodium taurodihydrofusidate (STDHF) and sodium glycocholate (GC) were obtained from Sigma (St. Louis, MO, U.S.A.) and California Biotechnology (Mountain View, CA, U.S.A.), respectively. Hydroxypropyl-β-cyclodextrin (HPβ CD) and Hypnorm were from Janssen (Beerse, Belgium). Radioactive ^{125}I was obtained from Amersham (Houten, The Netherlands). All other reagents used were of standard laboratory grade.

Results

Pharmacokinetics of Org2766 after i.v., s.c. and i.n. administration in rats

The Org2766 concentrations detected in rat serum after i.n. placebo administration were very low and in the range of the detection limit of the radioimmunoassay (Table 1). Thus, the cross-reactivity of the Org2766 antibody with possibly interfering endogenous compounds was negligible and the measured peptide serum concentrations were not corrected for endogenous immunoreactivity. After an i.v. bolus injection of 75 μg kg^{-1} of the ACTH(4–9) analogue an initial rapid decline in serum concentrations was followed by a slower phase of elimination (Figure 1). After 3 h the serum levels were still elevated. I.n. delivery of the same dose of the ACTH(4–9) analogue resulted in slowly increasing serum peptide concentrations with a peak concentration of 2.5 ng ml^{-1} at approximately 1 h post-administration. The absolute bioavailability of nasally administered Org2766 was found to be 19 ± 8% (Table 1). S.c. administered Org2766 at a dose of 15 μg kg^{-1} showed absorption peak serum levels at 30 min (Figure 1).

Table 1 Bioavailabilities of Org2766 following nasal and s.c. administration to rats

Dose ($\mu g\ kg^{-1}$)	*Route*	*Additive*	AUC_{0-180} ($ng\ ml^{-1}\ min$)	*F* (%)	n
0	i.n.	–	2 ± 1	0	(4)
25	i.n.	–	55.7 ± 18.1	13 ± 4	(4)
75	i.n.	–	253.9 ± 113.3	19 ± 8	(6)
250	i.n.	–	408.8 ± 121.7	13 ± 4	(6)
25	i.n.	DMβCD 5%	269.1 ± 85.4	65 ± 21	(8)
75	i.n.	DMβCD 5%	1033.5 ± 164.7	76 ± 12	(6)
250	i.n.	DMβCD 5%	2362.6 ± 610.0	63 ± 19	(6)
25	s.c.	–	ND	ND	
75	s.c.	–	940.0 ± 158.7	69 ± 12	(6)
250	s.c.	–	3519.6 ± 854.0	108 ± 26	(4)

The areas under the serum concentration-time curves measured up to 180 min post-administration (AUC_{0-180}) and the bioavailability (F) data are given as the mean ± s.d. of the number of animals given in parentheses (*n*). ND is not determined. *Significantly different from the s.c. 75 $\mu g\ kg^{-1}$ dose ($P<0.05$).

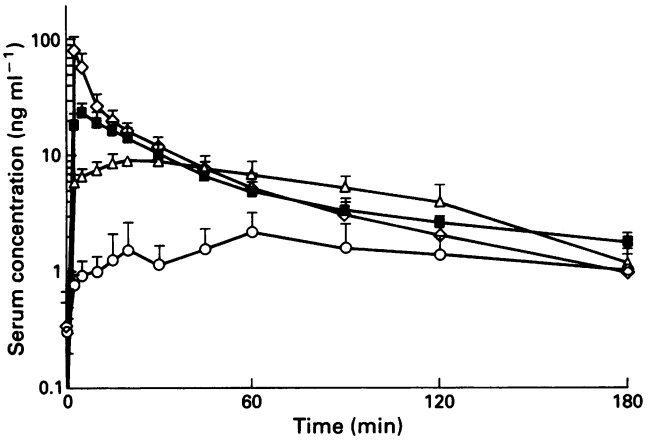

Figure 1 Mean ± s.d. serum Org2766 concentrations following administration of 75 $\mu g\ kg^{-1}$ Org2766 in rats: (◇) i.v.; (△) s.c.; (○) i.n. without enhancer; (■) i.n. with 5% (w/v) dimethyl-β-cyclodextrin. $n = 6$.

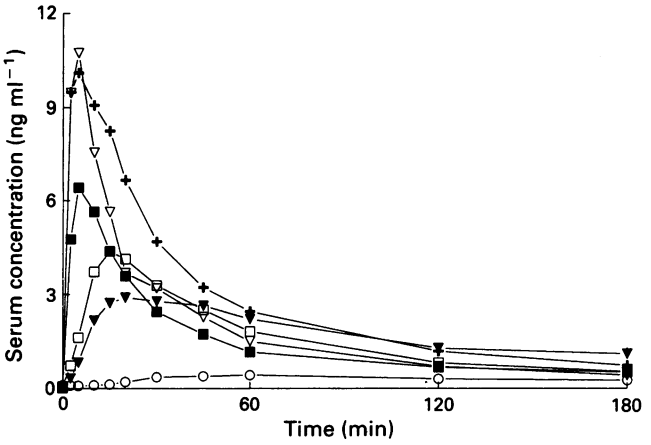

Figure 2 Mean serum Org2766 concentrations following nasal administration of a dose of 25 $\mu g\ kg^{-1}$ Org2766 with absorption enhancers in rats: (○) without enhancer; (▼) 5% α-CD; (□) 2% DMβCD; (■) 5% DMβCD; (▽) 0.5% STDHF; (+) 1% GC. See Table 2 for the number of rats used in the experiments. See text for abbreviations.

The peptide bioavailability after s.c. injection was 3 to 4 times as large as the nasal bioavailability (Table 1).

For i.n. and s.c. administered Org2766 at doses of 25 to 250 $\mu g\ kg^{-1}$ the AUC_{0-180} and the corresponding bioavailabilities are given in Table 1. The AUC's were proportional to the i.n. administered dose Org2766, indicating that the bioavailability values were not dependent on the peptide dose given. S.c. administered Org2766 was significantly better absorbed from a large 250 $\mu g\ kg^{-1}$ dose, than a smaller 75 $\mu g\ kg^{-1}$ dose.

Nasal delivery of Org2766 in rats: effect of absorption enhancers

A number of compounds were studied for their ability to improve nasal Org2766 absorption in rats. The serum concentration versus time curves obtained in these experiments are depicted in Figure 2 and the pharmacokinetic data are summarized in Table 2. Addition of HPβCD to the nasal peptide preparation (25 $\mu g\ kg^{-1}$) did not improve the absorption of the ACTH(4–9) analogue compared to administration of the peptide alone. All other compounds studied improved the nasal peptide absorption remarkably. The nasal bioavailabilities ranged from 70 ± 23% for (2% w/v) DMβCD to 118 ± 50% for (1% w/v) GC. Addition of 2 and 5% DMβCD resulted in a similar improvement in bioavailability. The times to reach the peak peptide serum concentrations were decreased by the effective enhancers. For DMβCD (5%), STDHF (0.5%) and GC (1%) the maximum peptide concentrations were already achieved at 5 min after administration. The nasal absorption was slower when α-CD (5%) and DMβCD (2%) were used as enhancers, showing times to reach the maximum serum concentration of 16 ± 2 and 27 ± 13 min (mean ± s.d.), respectively (Table 2).

Table 2 Nasal administration of Org2766 (25 $\mu g\ kg^{-1}$) with absorption enhancers in rats: pharmacokinetic parameters

Route	*Additive*	T_{max} (min)	C_{max} ($ng\ ml^{-1}$)	*F* (%)	n
i.v.	–			100 ± 31	(10)
i.n.	–	49 ± 7	0.4 ± 0.2	13 ± 4	(4)
i.n.	DMβCD 2%	16 ± 2*	4.5 ± 1.3*	70 ± 23*	(8)
i.n.	DMβCD 5%	6 ± 2*	6.8 ± 2.2*	65 ± 21*	(8)
i.n.	α-CD 5%	27 ± 13*	3.1 ± 0.4*	76 ± 17*	(4)
i.n.	HPβCD 5%	35 ± 21	1.0 ± 0.7	16 ± 4	(4)
i.n.	STDHF 0.5%	5 ± 0*	11 ± 1.1*	82 ± 24*	(4)
i.n.	GC 1%	5 ± 2*	11 ± 3.7*	118 ± 50*	(8)

The time at which the serum peak concentration was reached (T_{max}), the peak serum concentration (C_{max}), and the bioavailability (F) are given as mean ± s.d. of the number of animals given in parentheses (*n*). The statistical evaluation of the bioavailability data was performed on the raw AUC data (One way analysis of variance). *Significantly different from i.n. Org2766 without additives ($P<0.05$). For abbreviations, see text.

Reversibility of the enhancing effect of DMβCD on nasal Org2766 absorption

In order to determine the duration of the enhancing effect of DMβCD on nasal Org2766 absorption, the peptide (25 μg kg^{-1}) was given i.n. to rats 1 h after DMβCD instillation. The serum concentration versus time curves are shown in Figure 3. The serum-peptide concentrations appeared to be largely reduced compared to simultaneous administration of peptide and enhancer. The AUC values were decreased about 2 fold: $AUC_{0-60\,min}$ was 411 ± 127 ng ml^{-1} min for coadministration of peptide and enhancer, and $AUC_{0-60\,min}$ was 186 ± 94.2 ng ml^{-1} min when the peptide was given 60 min after DMβCD administration. Although the effect of DMβCD on Org2766 absorption was reversed substantially within 1 h, the nasal absorption had not yet completely returned to the level seen without any enhancer.

Nasal absorption of Org2766 with DMβCD in rabbits

In rabbits, nasal administration of 25 μg kg^{-1} Org2766 with 5% (w/v) DMβCD resulted in significantly higher Org2766 serum concentrations than administration of Org2766 alone during the first 45 min (Figure 4). Absolute bioavailability values of 10 ± 6% and 17 ± 8% were calculated for nasal administration of Org2766 without enhancer and with 5% (w/v) DMβCD, respectively (Table 3).

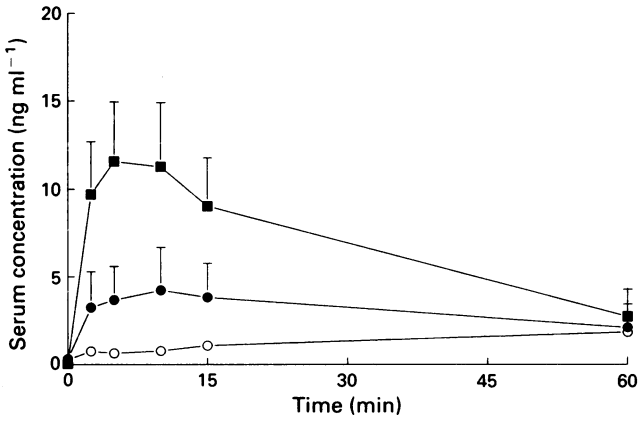

Figure 3 Mean ± s.d. serum Org2766 concentrations following nasal administration of 25 μg kg^{-1} Org2766 in rats: (○) without enhancer; (■) 5% dimethyl-β-cyclodextrin (DMβCD) administered simultaneously with Org2766; (●) 5% DMβCD given 1 h before Org2766 administration. $n = 3$ to 6 rats. The $AUC_{0-60\,min}$ following simultaneous administration of Org2766 and DMβCD was significantly different from peptide administration without enhancer and from peptide administration 1 h after DMβCD instillation ($P < 0.01$, one way analysis of variance).

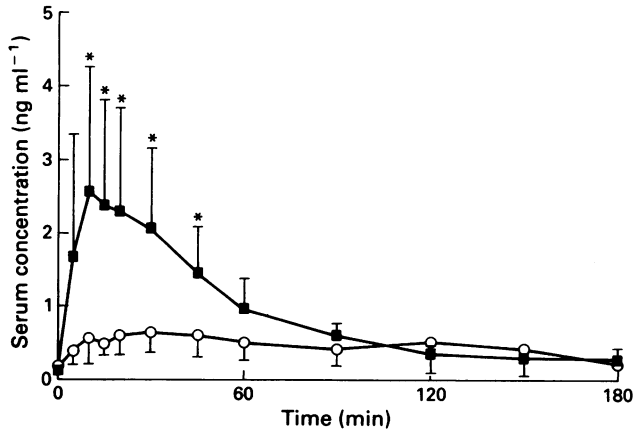

Figure 4 Mean ± s.d. serum Org2766 concentrations following nasal administration of 25 μg kg^{-1} Org2766 in rabbits: (○) without enhancer; (■) 5% dimethyl-β-cyclodextrin. $n = 7$ animals. *Significantly different from i.n. administration without enhancer ($P < 0.05$, t test for paired results).

Functional recovery from peripheral nerve damage after different routes of peptide administration

The efficacy of nasally administered Org2766 with DMβCD as an enhancer was studied in a rat model in which the functional recovery from a crush lesion in the sciatic nerve was determined with a foot reflex withdrawal test. Rats untreated with Org2766 started to respond to the electric stimulus in the withdrawal test at days 16 to 18, and were considered fully recovered at days 22 to 24. Rats receiving a s.c. dose of 75 μg kg^{-1} 48 h^{-1} Org2766 showed an accelerated recovery of nerve function compared to untreated rats (Figure 5). Since nasal dosing could only be performed reproducibly and quantitatively when the rats were anaesthetized just before peptide administration, the s.c. and i.v. treated animals were also anaesthetized. Hypnorm anaesthesia did not decrease the beneficial effect of s.c. administered Org2766 (Figure 6). I.n. and i.v. administrations of Org2766 at a dose of 75 μg kg^{-1} 48 h^{-1} were ineffective in accelerating the functional recovery from nerve damage compared to placebo treated animals. From Figure 6 it is evident that nasal peptide doses ranging from 0.75 to 375 μg kg^{-1} 48 h^{-1} did not improve the recovery rate compared to untreated rats.

Table 3 Nasal administration of Org2766 (25 μg kg^{-1}) with dimethyl-β-cyclodextrin (DMβCD, 5% w/v) as absorption enhancer in rabbits: pharmacokinetic parameters

Route	*Additive*	C_{max} (ng ml^{-1})	T_{max} (min)	*F* (%)	n
i.v.	–			100 ± 14	(7)
i.n.	–	0.8 ± 0.4	36 ± 39	10 ± 6	(7)
i.n.	DMβCD	2.9 ± 1.6*	15 ± 8	17 ± 8	(7)

The time at which the serum peak concentration was reached (T_{max}), the peak serum concentration (C_{max}) and the bioavailability (F) are given as mean ± s.d. of the number of animals given in parentheses (n). The statistical evaluation of the bioavailability data was performed on the raw AUC data (Student's t test for paired results). *Significantly different from i.n. Org2766 without additives ($P < 0.05$).

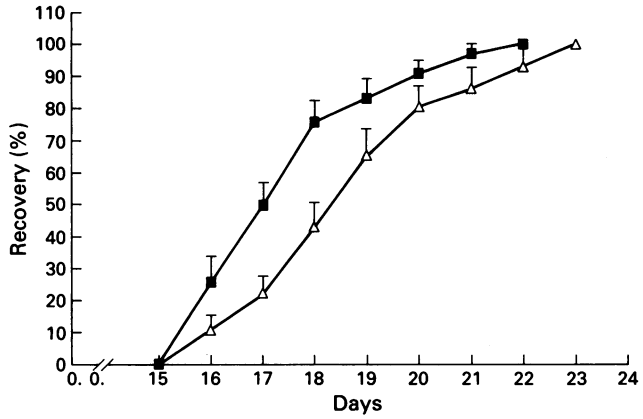

Figure 5 The effect of s.c. administered Org2766 (75 μg kg^{-1} 48 h^{-1}) on functional recovery following a crush lesion of the right sciatic nerve in rats: (△) placebo; (■) Org2766. The percentage recovery was determined by reflex withdrawal reaction on a local foot sole stimulation of 0.1–0.6 mA. The percentage recovery is plotted as the mean ± s.e.mean of 11 to 12 rats. The rats were anaesthetized.

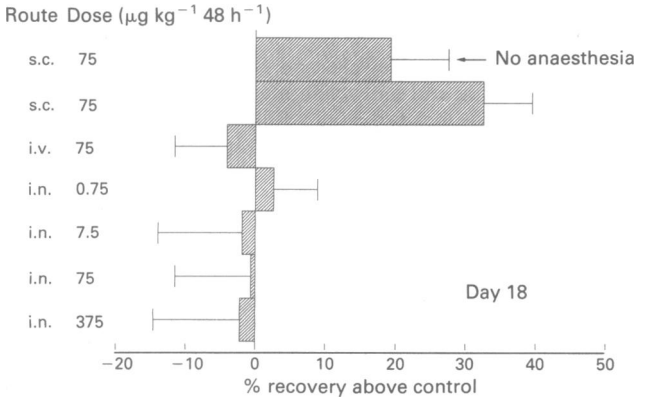

Figure 6 The effect of Org2766 on functional recovery following a crush lesion in the right sciatic nerve. The % recovery above control is the % recovery of peptide-treated animals subtracted from the % recovery of their own control group. The % recovery above control is plotted as the mean recovery ± s.e.mean of 11 to 12 rats per group on day 18 after the lesion. All rats were anaesthetized, with the exception of one s.c. dosed group. The statistical evaluation (Mann-Whitney) was performed on the raw data obtained at day 18, comparing the peptide treatment values of each experimental group with their respective saline-treatment values. Significantly enhanced absorption was only observed for s.c. administration of Org2766 with and without anaesthesia ($P<0.01$, and $P<0.05$, respectively).

Discussion

The present study showed that following nasal administration the ACTH(4–9) analogue, Org2766, was taken up in the systemic circulation in both rats and rabbits, the bioavailability being about 15% and 10%, respectively. The absorption in rats could be largely improved by addition of a number of absorption enhancers to the nasal preparation such as the cyclodextrins α-CD and DMβCD, the fusidate derivative STDHF, and the bile salt GC. Although many compounds of widely differing chemical nature have been shown to enhance nasal peptide absorption (Lee *et al.*, 1991), major problems encountered with many enhancers are their possible adverse effects on the nasal epithelium and mucociliary activity, and the occurrence or irritation. STDHF, for example, severely damages the nasal epithelium (Ennis *et al.*, 1990), decreases *in vitro* mucociliary activity at concentrations as low as 0.2% (w/v) (Gizurarson *et al.*, 1990; Hermens *et al.*, 1990), and is not well tolerated after nasal administration in man (Kissel *et al.*, 1992). GC which is an effective enhancer of many peptide and protein drugs (Pontiroli *et al.*, 1989), has less adverse effects (Ennis *et al.*, 1990; Gizurarson *et al.*, 1990; Hermens *et al.*, 1990). Previous studies in our laboratory have shown that GC and DMβCD at concentrations of 1 and 2% (w/v), respectively, had the same effect on *in vitro* ciliary activity (Schipper *et al.*, 1992a). The decrease in ciliary movement was much smaller than the influence of enhancers such as STDHF, sodium deoxycholate, sodium cholate, or laureth-9 (Hermens *et al.*, 1990). The cyclodextrin derivative DMβCD in concentrations up to 6% (w/v) was well tolerated when given intranasally as an oestradiol- or progesterone-cyclodextrin complex to female patients daily for a period of at least 6 months (Hermens *et al.*, 1992).

The high permeability of the nasal muscosa for Org2766 as caused by DMβCD returned to the normal physiological state in time. This was demonstrated by the decrease in effect of DMβCD on nasal Org2766 absorption when the peptide was not administered simultaneously with the enhancer but 1 h later. Comparable studies by Hirai *et al.* (1981) and Lee *et al.* (1988) showed that the absorption enhancer laureth-9 increased the permeability of rat nasal mucosa for a long period of time (>2 h), whereas the effects of GC and STDHF were, similar to DMβCD, limited in duration. Addition of DMβCD to the nasal peptide preparations improved the absorption in both the rat and rabbit animal model. The enhancement of absorption, however, was stronger in rats than in rabbits. Such species differences in the nasal absorption of peptides have been reported before (Baldwin *et al.*, 1990; Fisher *et al.*, 1991; Merkus *et al.*, 1993). Similar differences were also obtained for the nasal absorption of insulin with DMβCD. In rats the insulin absorption was almost complete, whereas in rabbits and man, insulin was hardly absorbed following delivery of nasal insulin/DMβCD solutions (Merkus *et al.*, 1991; Schipper *et al.*, 1993).

Several mechanisms of action by which cyclodextrins can improve nasal peptide absorption have been suggested (Irie *et al.*, 1992; Shao *et al.*, 1992). Cyclodextrins have been shown to interact with membrane lipids and proteins in the nasal epithelium, which may reduce the barrier function of the epithelium. Cyclodextrins are also able to inhibit proteolytic enzyme activity in the nasal mucosa (Irie *et al.*, 1992), and finally, they may act directly upon the peptide molecule thereby inhibiting peptide aggregation. (Shao *et al.*, 1992). In good agreement with the reported mechanisms of action, α-CD and DMβCD have been observed to influence strongly the nasal epithelial membrane barrier and enzyme activity, while HPβCD, ineffective in promoting nasal Org2766 absorption as evident from the present study, did not influence either the membrane barrier properties or the protease activities (Irie *et al.*, 1992; Shao *et al.*, 1992).

The effect of Org2766 on nerve recovery is highly dose-dependent. The dose-response relationship is of a so-called bell-shaped type. S.c. administration of Org2766 in doses of 7.5 or 75 μg kg^{-1} 48 h^{-1} facilitated peripheral nerve repair after a crush-lesion in the sciatic nerve, whereas low doses of 0.075, 0.75, and a high dose of 225 μg kg^{-1} 48 h^{-1} were completely ineffective (Gispen & Wiegant, 1990). In spite of the good absorption of nasally administered Org2766 with DMβCD, resulting in high serum peptide concentrations, the ACTH(4–9) analogue did not facilitate peripheral nerve regeneration after nasal delivery. The strong dose-dependency of the effect of Org2766, however, probably does not explain the negative results obtained with nasal administration. The serum Org2766 concentrations were in the same order of magnitude following nasal and s.c. delivery. Moreover, the dose given i.n. was varied between 0.75 and 375 μg kg^{-1} 48 h^{-1}. Thus, one would expect that at least one of these dosages would result in effective serum concentrations. Nevertheless, none of the nasal preparations used in the present study improved nerve repair. More likely, the serum concentration time-profile following nasal administration might be too rapid for the peptide to exert a beneficial effect. The serum concentration-time profile after nasal administration was quite similar to that found after i.v. injection. Although the initial serum concentration after i.v. injection was larger than the peak serum concentration obtained after i.n. delivery, they both declined very rapidly, and it is very striking that both i.n. and i.v. administration of the peptide did not result in improved functional recovery of the sciatic nerve. Following s.c. administration, which was the only effective route of delivery in the present experiments, the absorption phase was longer: the Org2766 peak serum concentrations were smaller, and did not decline as rapidly as observed with i.n. and i.v. routes of administration.

Changes in peptide binding to blood components, drug distribution and/or metabolism affected by the cyclodextrin derivative may also have influenced the amount of free serum ACTH(4–9) analogue, and, thus, the effect on peripheral nerve regeneration. This would, however, not explain the ineffectiveness of i.v. administered Org2766, since i.v. injections were performed in the absence of DMβCD. It has previously been suggested that in order to improve nerve regeneration Org2766 should be available repeatedly during a short, critical period of about 1 week following the crush (Edwards *et al.*, 1984; De Koning & Gispen, 1987; Gispen & Wiegant, 1990). The present data corroborate this view and

indicate that to facilitate nerve repair, significant amounts of the peptides must be present in the systemic circulation for an extended period of time as obtained with s.c. delivery, whereas the high but rapidly disappearing peak concentrations associated with i.v. and i.n. administration oppose the beneficial effect on peripheral nerve regeneration. The previous observation that continuous s.c. delivery of Org2766 using a depot microsphere preparation has resulted in improved nerve repair is in accordance with the present data (Gispen & Wiegant, 1990).

In conclusion, the ACTH(4–9) analogue Org2766 was absorbed following nasal administratiom in rats and rabbits. The absorption could be substantially improved by coadministration of absorption enhancers such as the cyclodextrin derivative, DMβCD. However, in spite of the large absorption, the peptide did not facilitate the functional recovery from peripheral nerve damage following nasal delivery in rats, probably due to the observed rapid absorption profile.

The technical assistance in the nerve recovery experiments of G. De Ruiter and M. Hali is greatly appreciated. We wish to thank Dr B. Naisbett for her careful evaluation of the manuscript.

References

BALDWIN, P.A., KLINGBEIL, C.K., GRIMM, C.J. & LONGENECKER, J.C. (1990). The effect of sodium tauro-24,25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models. *Pharm. Res.*, **7**, 547–552.

CHIEN, Y.W., SU, K.S.E. & CHANG, S.F. (1989). *Nasal Systemic Drug Delivery*. New York: Marcel Dekker.

DEKKER, A.J.A.M., PRINCEN, M.M., DE NIJS, H., DE LEEDE, L.G.J. & BROEKKAMP, C.L.E. (1987). Acceleration of recovery from sciatic nerve damage by the ACTH(4–9) analog Org2766: different routes of administration. *Peptides*, **8**, 1057–1059.

DE KONING, P., BRAKKEE, J.H. & GISPEN, W.H. (1986). Methods for producing a reproducible crush in the sciatic and tibial nerve of the rat and rapid and precise testing of return of sensory function: beneficial effect of melanocortins. *J. Neurol. Sci.*, **74**, 237–246.

DE KONING, P. & GISPEN, W.H. (1987). Org2766 improves functional and electrophysiological aspects of regenerating sciatic nerve in the rat. *Peptides*, **8**, 415–422.

EDWARDS, P.M., VAN DER ZEE, C.E.E.M., VERHAAGEN, J., SCHOTMAN, P., JENNEKENS, F.G.I. & GISPEN, W.H. (1984). Evidence that the neurotrophic actions of α-MSH may derive from its ability to mimic actions of a peptide formed in degenerating nerve stumps. *J. Neurol. Sci.*, **64**, 333–340.

ENNIS, R.E., BORDEN, L. & LEE, W.A. (1990). The effects of permeation enhancers on the surface morphology of the rat nasal mucosa: a scanning electron microscopy study. *Pharm. Res.*, **7**, 468–475.

FISHER, A.N., FARRAJ, N.F., O'HAGAN, D.T., JABBAL-GILL, I., JOHANSEN, B.R., DAVIS, S.S. & ILLUM, L. (1991). Effect of L-α-lysophosphatidylcholine on the nasal absorption of human growth hormone in three animal species. *Int. J. Pharm.*, **74**, 147–156.

GERRITSEN VAN DER HOOP, R., VECHT, C.J., VAN DER BURG, M.E.L., ELDERSON, A., BOOGERD, W., HEIMANS, J.J., VRIES, E.P., VAN HOUWELINGEN, J.C., JENNEKENS, F.G.I., GISPEN, W.H. & NEIJT, J.P. (1990). Prevention of cis-platin neurotoxicity with an ACTH(4–9) analogue in patients with ovarian cancer. *N. Engl. J. Med.*, **322**, 89–94.

GISPEN, W.H. & WIEGANT, V.M. (1990). Melanocortins in peripheral nerve regeneration: influence of dosage forms and routes of administration. *J. Controlled Rel.*, **13**, 203–211.

GIZURARSON, S., MARRIOTT, C., MARTIN, G.P. & BECHGAARD, E. (1990). The influence of insulin and some excipients used in nasal insulin preparations on mucociliary clearance. *Int. J. Pharm.*, **65**, 243–247.

HERMENS, W.A.J.J., BELDER, C.W.J., MERKUS, C.M.W.M., HOOYMANS, P.M., VERHOEF, J. & MERKUS, F.W.H.M. (1992). Intranasal administration of estradiol in combination with progesterone to oophorectomized women: a pilot study. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **43**, 65–70.

HERMENS, W.A.J.J., HOOYMANS, P.M., VERHOEF, J. & MERKUS, F.W.H.M. (1990). Effects of absorption enhancers on human nasal tissue ciliary movement in vitro. *Pharm. Res.*, **7**, 144–146.

HIRAI, S., YASHIKI, T. & MIMA, H. (1981). Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.*, **9**, 173–184.

IRIE, T., WAKAMATSU, K., ARIMA, H., ARITOMI, H. & UEKAMA, K. (1992). Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. *Int. J. Pharm.*, **84**, 129–139.

KISSEL, T., DREWE, J., BANTLE, S., RUMMELT, A. & BEGLINGER, C. (1992). Tolerability and absorption enhancement of intranasally administered octreotide by sodium taurodihydrofusidate in healthy subjects. *Pharm. Res.*, **9**, 52–57.

LEE, V.H.L., YAMAMOTO, A. & BHASKAR, U. (1991). Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug Carrier Syst.*, **8**, 91–192.

LEE, W.A., ENNIS, R.E. & FOSTER, L.C. (1988). Histological studies of insulin absorption across the nasal mucosa in the presence of sodium taurodihydrofusidate. *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, **15**, 70–71.

MERKUS, F.W.H.M., SCHIPPER, N.G.M., ROMEIJN, S.G. & VERHOEF, J. (1993). Absorption enhancers in nasal drug delivery: efficacy and safety. *J. Controlled Rel.*, **24**, 201–208.

MERKUS, F.W.H.M., VERHOEF, J., ROMEIJN, S.G. & SCHIPPER, N.G.M. (1991). Absorption enhancing effect of cyclodextrins in intranasally administered insulin in rats. *Pharm. Res.*, **8**, 588–592.

PONTIROLI, A.E., CALDERARA, A. & POZZA, G. (1989). Intranasal drug delivery: potential advantages and limitations from a clinical pharmacokinetic perspective. *Clin. Pharmacokinet.*, **17**, 299–307.

SCHIPPER, N.G.M., DE LANNOY, L.M., ROMEIJN, S.G., VERHOEF, J. & MERKUS, F.W.H.M. (1992a). Nasal administration of an ACTH (4–9) analogue in rats. In *Minutes on Buccal and Nasal Administration as an Alternative to Parenteral Administration*. ed. Duchêne, D. pp. 297–302. Paris: Éditions de Santé.

SCHIPPER, N.G.M., ROMEIJN, S.G., VERHOEF, J. & MERKUS, F.W.H.M. (1993). Nasal insulin delivery with dimethyl-beta-cyclodextrin as an absorption enhancer in rabbits: powder more effective than liquid formulations. *Pharm. Res.*, **10**, 682–686.

SCHIPPER, N.G.M., VERHOEF, J., ROMEIJN, S.G. & MERKUS, F.W.H.M. (1992b). Absorption enhancers in nasal insulin delivery and their influence on nasal ciliary functioning. *J. Controlled Rel.*, **21**, 173–186.

SHAO, Z., KRISHNAMOORTHY, R. & MITRA, K. (1992). Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluations. *Pharm. Res.*, **9**, 1157–1163.

SPRUYT, B., PITSIKAS, N., ALGERI, S. & GISPEN, W.H. (1990). Org2766 improves performance of rats with unilateral lesions in the fimbria fornix in a spatial learning task. *Brain Res.*, **527**, 192–197.

STRAND, F.L., ROSE, K.J., ZUCARELLI, L.A., KUME, J., ALVES, S.E., ANTONAWICH, F.J. & GARETT, L.Y. (1991). Neuropeptide hormones as neurotrophic factors. *Physiol. Rev.*, **71**, 1017–1046.

VAN DER ZEE, C.E.E.M., BRAKKEE, J.H. & GISPEN, W.H. (1988). α-MSH and Org2766 in peripheral nerve regeneration: different routes of delivery. *Eur. J. Pharmacol.*, **147**, 351–357.

VAN DER ZEE, C.E.E.M., BRAKKEE, J.H. & GISPEN, W.H. (1991). Putative neurotrophic factors and functional recovery from peripheral nerve damage in the rat. *Br. J. Pharmacol.*, **103**, 1041–1046.

WOLTERINK, G., VAN ZANTEN, E., KAMSTEEG, H., RADHAKISHUN, F.S. & VAN REE, J.M. (1990). Functional recovery after destruction of dopamine systems in the nucleus accumbens of rats. II. Facilitation by the ACTH(4–9) analog Org2766. *Brain Res.*, **507**, 101–108.

(Received June 8, 1993
Revised July 28, 1993
Accepted July 30, 1993)