MELANOCORTINS IN PERIPHERAL NERVE REGENERATION: INFLUENCE OF DOSAGE FORMS AND ROUTES OF ADMINISTRATION*

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Neutrophic peptides derived from the melanocortin (ACTH/MSH) family stimulate both in vitro and in vivo the outgrowth of neurites from developing neurones or damaged nerves. In medical biology the search for a new lead in the pharmacotherapy of brain and nerve lesions is tremendous as the incidence of especially aged related neurodegenerative diseases and nervous system trauma in a greying society is profound and currently little, if any, specific drug treatment seems to be at hand. In rats, the efficacy of melanocortins in accelerating functional recovery from sciatic nerve damage following various types of sub-cutaneous and oral administration was assessed. Rats bearing a crush lesion in the sciatic nerve and receiving melanocortins as a subcutaneous bolus injection every other day showed an accelerated recovery of function as tested in a foot withdrawal test or a walking pattern analysis. Sustained release from osmotic ALZET-minipumps or from lactate/glycolate biodegradable microspheres, both dosage forms implanted subcutaneously, were similarly effective. Although melanocortins have been shown to affect some CNS function following oral administration, both delivery via drinking water and via a stomach tube rendered the peptide ineffective in facilitating peripheral nerve repair. In the study on the pharmacotherapy of nerve trauma, local application of neurotrophic factors is both feasible as well as implicated by pathophysiological mechanisms governing post-lesion neurite outgrowth. In experiments concerning the repair of transected nerves we used microporous Accurel® polypropylene tubes to enclose the site of injury. The Accurel® can adsorb high quantities of peptides and this feature was used to deliver α -MSH at the repair site of sutured rat sciatic nerve. In studies on the crushed nerve, local application of the peptide was achieved by wrapping a peptide-impregnated biodegradable gelatine foam matrix (Willospan®) around the site of injury. Under both conditions local delivery leads to enhanced recovery of function following sciatic nerve damage. That local delivery and not leakage from the local site via systemic routes facilitated the recovery process was evident from control studies where the gelatine foam was wrapped around the contralateral nerve and no facilitated recovery could be established in the ipsilateral crushed nerve. Although currently there is little insight into the actual amount of peptide reaching the site of injury via the different routes of administration, it seems appropriate to conclude that a peak amount of peptide must be available in a short period following the nerve damage to facilitate the repair process. In the clinic different routes may be selected depending upon the nature of the nerve damage, i.e. mechanical trauma (local, biodegradable) or intoxication (bolus injection or biodegradable microspheres).

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INTRODUCTION

Melanocortins are peptides related to the pituitary hormones corticotropin (ACTH) and melanotropin (MSH), and as hormones they mediate a variety of adaptive responses, a phenomenon well preserved throughout the vertebra phyla. A fundamental aspect of the neuropeptide concept, as formulated by De Wied [1], is that pituitary-borne peptides may reach nervous system structures and thus directly modulate nervous system function and behavior. Considering neural tissue as a target for circulating peptides one wonders what, if any, similarity there is between the mechanism of action of such peptides in the nervous system and in their peripheral target cells. In addition to their induction of target-specific responses, peptide hormones are known to exert trophic influences in their target organs which involve enhanced blood supply, increased oxygen and energy consumption and macromolecule synthesis [2]. Indeed, in neural tissue, in addition to a variety of tissue specific [3] and behavioral effects (for review see Ref. [4]), melanocortins were shown to influence the synthesis of RNA and protein [5]. Historically this line of evidence prompted various groups of investigators to assess the efficacy of neuropeptide treatment in the therapy of post lesion repair in the nervous system. For it was argued that under impoverished conditions the target cell would be especially in need of trophic support. Early studies of the effect of melanocortins on recovery from central nervous system (CNS) lesions gave conflicting results (for review see Ref. [6]). Peptides with full endocrine activity were used, since the rationale behind the treatment was — in contrast to our present views — to reduce, via stimulation of the release of corticosteroids, the formation of reactive scar tissue, which was believed to limit proper neural tissue

regeneration. However, the studies on the peripheral nervous system (see below) have shown that the immunosuppressant and corticotropic activities are not involved in the trophic response and certainly may have disturbed the final outcome of previous studies. Hormonally inactive fragments of melanocortins appeared to facilitate nerve repair following mechanical damage in animal models that measure recovery in functional, electrophysiological and histological parameters (for reviews see Refs. [2] and [7]). The peptide with the most promising clinical potential is the ACTH-(4–9) analog H-Met(O₂)-Glu-His-Phe-[D]-Lys-Phe (Org 2766), as oral or systemic treatment of animals and humans was shown to produce no toxic effects [8] and the peptide is protected against breakdown [9]. In the present paper we shall review our knowledge on the effects of different routes and dosage forms of melanocortins on their neurotrophic efficacy in the peripheral nervous system in the rat.

MATERIALS AND METHODS

Animals and surgery

Female rats of an inbred Wistar strain (Cpb-Wu, TNO, Zeist, The Netherlands), weighing 120–140 g, were used. The animals were housed in Makrolon cages (5 rats per cage) and maintained on a 12 h:12 h light:dark cycle, with food and water ad libitum. A crush lesion was made in the right sciatic nerve under Hypnorm[®] anaesthesia (Duphar, Weesp, The Netherlands; 0.8 ml per kg body weight) as described in detail by De Koning et al. [10]. Complete nerve transection followed by perineural suturing and placement of a microporous delivery system

around the nerve was performed as described in detail by Kuiters et al. [11] and Edwards et al. [12].

Functional recovery

The return of sensory motor function was measured by the reflex withdrawal of the foot sole from local noxious stimulation [12,13] or from a hot air stream [14,15]. The investigator performing the functional recovery tests was not aware of the treatment a given rat had received. The treatment code was partially broken at the end of the experiment to allow the analysis of data. The final treatment code was broken only after the analysis of the data was complete. Depending on the experimental design, group differences were analyzed with a χ^2 test or an analysis of variance for repeated measures followed by a supplemental *t*-test.

Peptides, routes and dosage forms

All peptides were synthesized and kindly donated by Organon International B.V. (Oss, The Netherlands).

Peptide administration

Subcutaneous (s.c.) injections of α -MSH or Org 2766, dissolved in 0.5 ml of saline (0.9% NaCl), were placed in the neck region. The dose tested was 75 μ g per kg body weight. The first injection was given immediately after the sciatic nerve was crushed and was repeated every 48 h. Control rats received 0.5 ml of saline every 48 h.

Osmotic ALZET 2002 mini-pumps (length 15 mm, volume 250 μ l, pumping rate 18 μ l per day; ALZA Corp., London, U.K.) were implanted s.c. in the neck region immediately following the crush procedure. The pumps were filled with Org 2766 in 250 μ l of saline and gave a constant release of 5 or 120 μ g/day [13] or 20, 30 or 40 μ g/day [15]. Control pumps were filled with saline. The pumps were removed under anaes-

thesia two weeks after implantation.

Biodegradable microspheres, consisting of a 1:1 copolymer of lactic and glycolic acids containing 3% (w/w) of Org 2766, were obtained from Organon International B.V. Forty mg of microspheres (peptide release was 40 μ g/day for 14 days *in vitro*) was suspended in 0.5 ml of an isotonic mannitol solution containing 0.05 μ g/ml of benzalkonium chloride, and the suspension was injected s.c. immediately following surgery. Control rats received microspheres loaded with vehicle [13,15].

Oral administration

Org 2766 was dissolved in drinking water. Rats received either 5 or 25 mg of Org 2766 in 25 ml of water per rat per day [13] or 1.5, 4.5 or 20 mg/22 ml [15]. A fresh solution was made every day. Control rats received normal drinking water. In another experiment rats received 1.5 or 15 mg/day [15] or 2 mg/day [13] Org 2766 in 1 ml of tap water, administered orally via a stomach tube. Treatment in both cases began immediately after the surgery and lasted throughout the experimental period.

Local application

Crush lesion

Peptide was applied locally by implanting a biodegradable matrix around the site of the crush lesion. The matrix (Willospoon 10 $\times$ 10 $\times$ 1 mm, gelatin foam) was impregnated with peptide by soaking the matrix in 100 μ l of saline containing 20 μ g of Org 2766 at room temperature for 5 min. The matrix used for implantation contained a total of 14 μ g of Org 2766. The peptide content was reduced to 50% after 5 min, and after 1 h the peptide content was only 10% of the original amount [13].

Nerve transection

The impregnation of Accurel[®] polypropylene tubing with α -MSH has been described in detail by Edwards et al. [12]. The microporous

Accurel polypropylene tubes used to enclose the transected nerves released *in vitro* a relatively constant value of about 1 $\mu\text{g}/\text{ml}$ for the first 7 days, declining slowly to 0.2–0.3 $\mu\text{g}/\text{ml}$ by day 14.

Radioimmunoassay of Org 2766

Radioimmunoassays (RIAs) employed a rabbit antiserum (K4) raised against bovine-thyroglobulin-conjugated Org 2766 (final dilution 1:8000), ^{125}I -labelled Org 2766 as tracer (ca. 10,000 cpm) and Org 2766 as standard peptide. The sensitivity of the assay (at 10% displacement of tracer) was 1 pg per tube. The following cross-reactivity data were obtained (expressed on mass basis): [Met(O₂)⁴, D-Lys⁸, Phe⁹]ACTH-(4–9) (Org 2766), 100%; [D-Lys⁸, Phe⁹]ACTH-(4–9), 0.85%; [Acetyl-Met(O₂)⁴, D-Lys⁸, Phe⁹]ACTH-(4–9), 1.6%; [D-Lys⁸, Phe⁹]ACTH-(5–9), 50%; [D-Lys⁸, Phe⁹]ACTH-(6–9), 62%; [D-Lys⁸, Phe⁹]ACTH-(7–9), 6.4%; ACTH-(4–10), 0.001%. RIAs were performed in duplicate on 1–100 μl aliquots of plasma in a final volume of 200 μl and were conducted in essence as described previously [16]. Plasma dilution curves paralleled the RIA standard curve.

RESULTS AND DISCUSSION

Effect of repeated injection

Rats bearing a crush lesion in the sciatic nerve and receiving Org 2766 or α -MSH at a dose of 75 $\mu\text{g}/\text{kg}/48\text{ h}$ showed an accelerated recovery of function as tested in the reflex withdrawal tests [10,13–15]. Strand and Kung [17] and Saint-Côme and Strand [18] reported in similar test situations the effects of daily s.c. administration of peptides. This facilitation of the recovery process is highly dose-dependent. The dose–response relationship is of the so-called inverted U-shape or bell-shape type. Org 2766 in a dose of 7.5 or 75 $\mu\text{g}/(\text{kg } 48\text{ h})$ s.c. was effective, whereas doses of 0.075, 0.75 or 225 $\mu\text{g}/$

(kg 48 h) s.c. were not (Fig. 1; 10 rats). Detailed structure–activity studies demonstrated that the neurotrophic activity of ACTH/MSH-like peptide is confined to the N-terminus of the molecule somewhere in the region (4–10) [15,19,20]. Although initially it was presumed that the melanotrophic rather than the corticotrophic features of the molecule were crucial for the neurotrophic effect [14], presently it seems that such a distinction is not tenable [20].

Another aspect that was studied using repeated subcutaneous injections was the question of when the peptide should be administered following surgery and for how long. It was established that the peptide must be administered during the first week post crush lesioning of the rat sciatic nerve in order to be effective. Continuation of treatment is not essential [21,22]. In fact, Saint-Côme and Strand [18] reported that treatment over too long a period was even unfavourable. If the treatment does not commence immediately after the surgery, i.e. on the day of surgery, but starts after a delay of only one week, the treatment is not effective [21]. Thus, there is a critical period following the lesioning during which the repair process is sensitive to peptide treatment. This is an important observation which has been discussed elsewhere in the context of the presumed mechanism of action of the peptide [2,23,24].

Effect of mini-pump delivery

Rats bearing a crush lesion in the sciatic nerve and receiving peptides from an osmotic mini-pump were tested for functional recovery using a foot reflex withdrawal test. Dekker et al. [15] reported enhanced recovery at delivery of Org 2766 at a rate of 20, 30 or 40 μg per day for 14 days. All three dosages shortened the recovery period by ca. 12–16%. In the study by Van der Zee et al. [13] no effect on the return of sensory motor function was observed at a release of 5 μg of Org 2766 or α -MSH per day. However, a significant acceleration of functional recovery was

Fig. 1. Return of sensory motor function as tested with the foot reflex withdrawal test. Indicated is the percentage of animals that show full sensory recovery at day 11 after crush. Org 2766 was administered by repeated s.c. injection in different doses (10 μg per rat equals 75 $\mu\text{g}/\text{kg}$). Ten rats per group; * denotes statistical significance from saline group.

demonstrated at an Org 2766 release of 120 μg per rat per day. Extrapolation of the dose level to a daily amount of peptide per kg body weight yielded an approximate effective dose of 225 $\mu\text{g}/\text{kg}$ [15] or 900 $\mu\text{g}/\text{kg}$ [13] per day. A distinct disadvantage of mini-pump delivery is that, once empty, the mini-pump must be removed from the rat. In chronic studies the repeated placement of mini-pumps might in itself then represent an unwanted procedural drawback. Therefore the neurotrophic efficacy of peptide treatment was also tested following the subcutaneous implantation of a biodegradable slow release preparation of the peptides.

Effect of injections with microspheres

Both Dekker et al. [15] and Van der Zee et al. [13] reported neurotrophic activity following a single s.c. injection with biodegradable polyglycolate-lactate microspheres, causing in both cases a constant release of 40 μg of Org 2766/day. Extrapolation of the dose to the amount of peptide per kg of rat per day yielded an approximate dose of 300 $\mu\text{g}/\text{kg}$. This biodegradable delivery system may have great importance to eventual clinical use of the peptide, especially in the protection from or counterac-

tion of chemically inflicted neuropathies. Thus, in animal models of chemotherapy induced neuropathies [8,25] or diabetes mellitus neuropathy [20], repeated subcutaneous injections of Org 2766 were highly effective in delaying or ameliorating the neuropathy. If such peptide treatment was to be effective in the clinic — for cisplatin neurotoxicity that seems indeed to be the case (see below) — then delivery via a biodegradable slow release preparation seems the preferable route of administration.

Oral administration

Oral administration of Org 2766 via the drinking water which rats consumed ad libitum (up to 22 or 25 ml per day) did not facilitate the functional recovery following sciatic nerve damage when tested in the range of 1.5 to 20 mg per day [15] or from 5 to 25 mg per day [13]. Extrapolation of the highest dose yielded a dose of approximately 200 mg/kg body weight per day. Both studies reported also the ineffectiveness of forced oral administration via a stomach tube of doses ranging from 1.5 to 15 mg/ml per day. Extrapolation of the highest dose tested

yielded a daily oral injection of 115 mg/kg body weight [13–15].

In other studies with ACTH/MSH-like peptides, oral administration was shown to be as effective as subcutaneous treatment provided the dose is at least 1000 times that required for subcutaneous injection. Rígter et al. [26] demonstrated that oral administration of a tablet containing 0.01–1 mg of Org 2766 reduced CO₂-induced amnesia for a passive avoidance response. Wolterink et al. [27] reported that oral injection via a stomach tube of 2 mg of Org 2766 per rat per day (compare to data obtained by Van der Zee et al. [13]) facilitated behavioural recovery following lesions to the nucleus accumbens in the rat. In the studies on oral administration, the minimal effective dose after subcutaneous administration was multiplied by 1000–5000 and possible breakdown of Org 2766 in the drinking water was negligible [15]. It is concluded that oral administration of Org 2766 is not an effective route for neurotrophic action on peripheral nerve function.

BIOAVAILABILITY

In view of the scant knowledge about the bioavailability of Org 2766 following the var-

ious routes and forms of administration, it is extremely difficult to estimate the actual amount of intact peptide in the circulation at various times following administration. It is even more difficult to estimate the final amount that reaches the site of action. These parameters will undoubtedly vary among different routes of administration. Hence we have begun to study the plasma concentration of Org 2766 by radioimmunoassay following different routes of administration in rats. In the first experiment we studied the plasma concentration over time following a single s.c. injection of 75 µg/kg of Org 2766 or mini-pump delivery of 120 µg per day. These dosages were previously shown to be effective in the functional recovery test [13]. As can be seen in Fig. 2A, 1 h following the s.c. administration approximately 1.5 ng/ml of immunoreactive Org 2766 (IR-Org 2766) was present in plasma. IR-Org 2766 levels rapidly declined over the following hours, and were below detection at 48 h after administration of the peptide. The osmotic mini-pump delivered a constant plasma level of approximately 2 ng/ml over the first 6 days following implantation (Fig. 2B). Previous experiments using radiolabelled peptide and proper electrophoretic separation of peptides suggested relatively high plasma levels of intact peptide shortly follow-

Fig. 2. Plasma concentration of IR-Org 2766 in rats at various time points following a single subcutaneous injection of 10 µg of Org 2766 (panel A), or implantation of an osmotic mini-pump loaded with 1680 µg of Org 2766 in 250 µl of saline (panel B). Closed symbols represent Org-treated animals, open symbols saline-treated controls. The dotted line indicates the detection limit of the radioimmunoassay. Data are presented as means of three separate determinations.

ing an s.c. injection of Org 2766 in rats. The elimination phase of Org 2766 was bi-exponential with an initial half life of 4 min [28]. Currently, the data are interpreted to suggest that, to improve regeneration, steady state plasma level of Org 2766 are not necessary, but peak amounts of peptide must be available repeatedly in a short, critical period following the crush. To obtain similar peak levels, higher doses (as calculated per rat per day) are required for the gradual release from a mini-pump than those needed to be effective after a bolus injection [13,15].

Local application

Although in all the studies on melanocortins and peripheral nerve regeneration the peptides were administered subcutaneously in the neck [2,7], the initial site of action could be at the site of the injury and outgrowth of the nerve [23]. If this is the case then peptide application directly at the site of injury would be predicted to accelerate recovery.

Crush lesion

Three groups of rats were used, all bearing a crush lesion at the right sciatic nerve. The biodegradable gelatin foam matrix, containing ca. 10–14 μg of Org 2766 or saline was wrapped around the site of injury. In one group, however, the peptide-containing foam was wrapped around the contralateral, intact sciatic nerve. The wrapping of peptide-containing foam around the injury site proved to facilitate the recovery of function whereas placement around the contralateral nerve was ineffective. Thus, although biodegradation of the matrix might in principle also release the peptide into the circulation, the amount that is released is so low that the crushed nerve is not facilitated in its recovery function. The effect of this form of administration may at best be compared to the effect of a single injection at the site of injury.

The foam is rapidly degraded, and in control experiments 1 h following placement only 10% of the original peptide could be recovered [13].

Nerve transection

In another study microporous Accurel polypropylene tubes were used to enclose the repair site of transected and sutured rat sciatic nerves [12]. Microporous Accurel can adsorb high quantities of peptides, and this phenomenon was used to deliver α -MSH at the site of injury. From control experiments it was concluded that the α -MSH concentration in the luminal fluid reached a relatively constant value of about 1 $\mu\text{g}/\text{ml}$ for the first 7 days. Recovery of the sensitivity of the foot sole following transection, suturing and placement of the Accurel tube was markedly facilitated when the Accurel had been impregnated with α -MSH [12]. This observation was extended in a study by Kuiters et al. [11] demonstrating improved motor nerve conduction velocities of transected, sutured nerves bearing an α -MSH-releasing Accurel tube around the site of injury.

In addition to the fact that the improvement of recovery following local application suggests that melanocortins may act at the site of injury [23], these data open the possibility of applying peptides locally at the site of injury in human nerve repair surgery. Such an approach combines advanced microsurgical repair with effective pharmacotherapy. The development of biodegradable polymeric tubes with peptide-binding characteristics similar to those of Accurel seems warranted.

CONCLUSIONS

Following the successful use of melanocortins in animal studies on recovery of function after peripheral nerve trauma, these peptides were studied in animal models of various neuropathies (Acrylamide intoxication [29]; cis-

platin intoxication [8]; diabetic neuropathy [20]). Again, peptide treatment markedly improved the function of the afflicted nerves or postponed the onset of the experimentally induced neuropathy. Hence, peptides like α -MSH or Org 2766 may be of great potential to the peripheral nerve clinic. It has been postulated that the generality of their effect suggests that there must be an endogenous peptide signal early in the trophic repertoire of the nerve (neuron) that is involved in the response of the nerve to counteract the influence of noxious stimuli [2].

To optimize eventual clinical application, more knowledge about the bioavailability and metabolism following various routes of administration is a necessary prerequisite. Further experiments are being carried out to address this issue.

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REFERENCES

- 1 D. de Wied, Effects of peptide hormones on behavior, in: W.F. Ganong and L. Martini (Eds.), *Frontiers in Neuroendocrinology*, Oxford University Press, London, 1969, pp. 97-140.
- 2 W.H. Gispen, P. de Koning, R.R.F. Kuiters, C.E.E.M. van der Zee and J. Verhaagen, On the neurotrophic properties of melanocortins, in: E.R. de Kloet, V. Wiegant and D. de Wied (Eds.), *Neuropeptides and Brain Function*, Prog. Brain Res., 72, 1987, 319-331.
- 3 W.H. Gispen, On the neurochemical mechanism of action of ACTH, Prog. Brain Res., 53 (1980) 193-206.
- 4 D. de Wied and J. Jolles, Neuropeptides derived from proopiomelanocortin: behavioral, physiological and neurochemical effects, Physiol. Rev., 62 (1982) 976-1059.
- 5 A.J. Dunn and P. Schotman, Effects of ACTH and related peptides on cerebral RNA and protein synthesis, Pharmacol. Ther., 12 (1981) 353-372.
- 6 M. Berry, J. Knowles, P. Willis, A.C. Riches, G.P. Morgans and D. Steers, A reappraisal of the effect of ACTH on the response of the central nervous system to injury, J. Anat., 128 (1979) 859-871.
- 7 F.L. Strand, K.J. Rose, J.A. King, A.C. Segarra and L.A. Zuccarelli, ACTH modulation of nerve development and regeneration, Prog. Neurobiol., 33 (1989) 45-85.
- 8 P. de Koning, J.P. Neijt, F.G.I. Jennekens and W.H. Gispen, Org 2766 protects from cisplatin-induced neurotoxicity in rats, Exp. Neurol., 97 (1987) 746-750.
- 9 H.M. Greven and D. de Wied, The influence of peptides derived from corticotrophin (ACTH) on performance. Structure activity studies, Prog. Brain Res., 39 (1973) 429-442.
- 10 P. de Koning, J.H. Brakkee and W.H. Gispen, 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 effects of melanocortins, J. Neurol. Sci., 74 (1986) 237-246.
- 11 R.R.F. Kuiters, P. de Koning, C.E.E.M. van der Zee, J.H. Brakkee, F.G.I. Jennekens and W.H. Gispen, Peripheral nerve repair in the rat: functional and electrophysiological aspects, Neuro Orthop., 6 (1988) 7-12.
- 12 P.M. Edwards, R.R.F. Kuiters, G.J. Boer and W.H. Gispen, Recovery from peripheral nerve transection is accelerated by local application of α -MSH by means of Accurel polypropylene tubes, J. Neurol. Sci., 74 (1986) 171-176.
- 13 C.E.E.M. van der Zee, J.H. Brakkee and W.H. Gispen, α -MSH and Org 2766 in peripheral nerve regeneration: different routes of delivery, Eur. J. Pharmacol., 147 (1988) 351-357.
- 14 W.A. Bijlsma, P. Schotman, F.G.I. Jennekens, W.H. Gispen and D. de Wied, The enhanced recovery of sensorimotor function in rats is related to the melanotropic moiety of ACTH/MSH neuropeptides, Eur. J. Pharmacol., 92 (1983) 231-236.
- 15 A.J.A.M. Dekker, M.M. Princen, H. de Nijs, L.G.J. de Leede and C.L.E. Broekkamp, Acceleration of recovery from sciatic nerve damage by the ACTH₄₋₉ analog Org 2766; different routes of administration, Peptides, 8 (1987) 1057-1059.
- 16 J. Barna, C.G.J. Sweep, H.D. Veldhuis and V.M. Wiegant, Differential effects of cisterna magna cannulation on β -endorphin levels in rat plasma and cerebrospinal fluid, Acta Endocrinol., 117 (1988) 517-524.
- 17 F.L. Strand and T.T. Kung, ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve, Peptides, 1 (1980) 135-138.
- 18 C. Saint-Côme and F.L. Strand, ACTH₄₋₉ analogue (Org 2766) improves qualitative and quantitative aspects of motor nerve regeneration, Peptides, 8 (Suppl.) (1988) 215-221.
- 19 W.A. Bijlsma, F.G.I. Jennekens, P. Schotman and W.H. Gispen, Effects of corticotrophin (ACTH) on recovery of sensorimotor function in the rat: struc-

- ture-activity study, *Eur. J. Pharmacol.*, 76 (1981) 73-79.
- 20 C.E.E.M. van der Zee, Neurotrophic peptides in animal models of peripheral nerve regeneration and diabetic neuropathy, Ph.D. Thesis, University of Utrecht, Utrecht, 1989.
 - 21 P.M. Edwards, C.E.E.M. van der Zee, J. Verhaagen, P. Schotman, F.G.I. Jennekens and W.H. Gispen, 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 (1984) 333-340.
 - 22 P. de Koning and W.H. Gispen, Org 2766 improves functional and electrophysiological aspects of regenerating sciatic nerve in the rat, *Peptides*, 8 (1987) 415-422.
 - 23 P.M. Edwards and W.H. Gispen, Melanocortin peptides and neural plasticity, in: J. Traber and W.H. Gispen (Eds.), *Senile Dementia of Alzheimer Type*, Springer Verlag, Berlin, 1985, pp. 231-240.
 - 24 P.R. Bär, L.H. Schrama and W.H. Gispen, Neurotrophic effects of ACTH/MSH-like peptides in the peripheral nervous system, in: D. de Wied (Ed.), *Neuropeptides, Basics and Perspectives*, Elsevier, Amsterdam, 1990, pp. 175-211.
 - 25 R. Gerritsen van der Hoop, P. de Koning, J.P. Neijt, F.G.I. Jennekens and W.H. Gispen, Efficacy of the neuropeptide Org 2766 in the prevention and treatment of cisplatin-induced neurotoxicity in rats, *Eur. J. Cancer Clin. Oncol.*, 24 (1988) 637-642.
 - 26 H. Rigter, R. Janssens-Elbertse and H. van Riezen, Reversal of amnesia by an orally active ACTH(4-9) analog Org 2766, *Pharmacol. Biochem. Behav.*, 5 (Suppl.1) (1976) 53-58.
 - 27 G. Wolterink, E. van Zanten, H. Kamsteeg, F.S. Radhakishun and J.M. van Ree, Functional recovery after destruction of dopamine systems in the nucleus accumbens of rats. II: Facilitation by the ACTH₄₋₉ analog Org 2766, *Brain Res.*, 507 (1990) 101-108.
 - 28 J. Verhoef and A. Witter, *In vivo* fate of a behaviorally active ACTH 4-9 analog in rats after systemic administration, *Pharmacol. Biochem. Behav.*, 4 (1976) 583-590.
 - 29 E. Sporel-Özkat, P.M. Edwards, R. Gerritsen van der Hoop and W.H. Gispen, An ACTH 4-9 analog, Org 2766 improves recovery from acrylamide neuropathy in rats, *Eur. J. Pharmacol.*, (1990) in press.

1. INTRODUCTION

Protease inhibitors and penetration enhancers are normally considered to circumvent the enzymatic and penetration barriers to peptide and protein absorption from mucosal routes of administration. Because of such barriers, the bioavailability of peptide and protein drugs from

mucosal routes is poor. For leuprolide this ranges from 0.06% following oral administration to 34% following vaginal administration [1]; for insulin the corresponding figures are 0.03% and 18% [2,3]. This paper will review the experience with protease inhibitors and penetration enhancers to date, address concerns with their use on a routine basis, and examine some alternative approaches to promote mucosal peptide and protein absorption. The emphasis will be on the nasal, buccal, rectal, and vaginal routes.

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