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Putative neurotrophic factors and functional recovery from peripheral nerve damage in the rat

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1 In rats, recovery of sensory-motor function following a crush lesion of the sciatic or tibial nerve was monitored by measuring foot reflex withdrawal from a local noxious stimulation of the foot sole.

2 Putative neurotrophic compounds were tested on this functional recovery model: melanocortins (peptides derived from ACTH (corticotropin) and α-MSH (melanotropin)), gangliosides and nimodipine were effective whereas isaxonine and TRH (thyrotropin releasing hormone) were not.

3 Structure-activity studies with melanocortins revealed a similar effectiveness of α-MSH, [N-Leu^4, D-Phe^7]-α-MSH, desacetyl-α-MSH and the ACTH$_{4-9}$ analogue ORG 2766, questioning the validity of the previously suggested notion that the melanotrophic properties of these peptides are responsible for their neurotrophic effect.

4 As recovery of function after peripheral nerve damage follows a similar time course in hypophysectomized (five days post operation) and sham-operated rats, effective melanocortin therapy does not mimic an endogenous peptide signal in the repair process from pituitary origin.

5 Subcutaneous treatment with ORG 2766 (7.5 μg kg^{-1} 48 h^{-1}) facilitates recovery of function following peripheral nerve damage in young (6–7 weeks old), mature (5 month old) and old (20 month old) rats.

6 In view of the diversity in structure of the effective neurotrophic factors and the complexity of nerve repair, the present data support the notion that peripheral nerve repair may be facilitated by different humoral factors likely to be active on different aspects of the recovery process.

Keywords: Peripheral nerve regeneration; sciatic crush lesion; adrenocorticotrophic hormone (ACTH); melanotropin (α-MSH); ORG 2766; neurotrophic factors; gangliosides; nimodipine

Introduction

Although progress has been made in the surgical repair of peripheral nerve trauma, it is clear that the development of new pharmacotherapeutics is a prerequisite for further advancement of repair of damaged nerves (Gelijns *et al.*, 1987). For many years neurologists turned to vitamin B treatment to improve peripheral nerve function with questionable results (Jennekens, 1984). Following the initial discovery of Nerve Growth Factor (NGF), more and more protein- and peptide-factors have been isolated and characterized that enhance neurite outgrowth in cultured foetal neurones (Varon, 1985; Dekker *et al.*, 1987a). As the molecular processes that underlie postlesion axonal repair in the adult in part resemble a replay of those processes that govern neurite outgrowth and network formation in the developing nervous system (Bär *et al.*, 1990), developmentally active factors are often screened for their efficacy in nerve repair. However, until now no clinically proven new pharmacotherapy has emerged. In the present study using a crush lesion of the rat sciatic nerve we evaluated the efficacy of various factors claimed to have beneficial effects on functional recovery in experimental regeneration and reinnervation. Although these factors are of a completely different chemical nature and would affect the neurone via different mechanisms of action, they have all been shown to accelerate maturation and neurite outgrowth in cultured foetal neurones (corticotropin/melanotropin (ACTH/MSH) like peptides: rat spinal cord and cerebral neurones, Richter-Landsberg *et al.*, 1987, Van der Neut *et al.*, 1988; gangliosides: various primary neurone cultures, see Ledeen 1984; nimodipine: medial raphe 5-hydroxytryptaminergic neurones, Azmitia, 1989; isaxonine: mouse spinal ganglion, Hugelin *et al.*, 1977). In the present experiments, detailed structure-activity studies were performed to characterize further the active site in ACTH/MSH-like peptides that may be responsible for their neurotrophic activity. The data show that not all factors that are reported to promote neurite outgrowth *in vitro* are effective in an *in vivo* nerve repair model and that there are multiple message sites encoding for the modulating influence of ACTH/MSH-like peptides on functional recovery following peripheral nerve lesion.

Methods

Animals and surgery

Female rats of an inbred Wistar strain (TNO CpB, Zeist, The Netherlands), 6–7 weeks of age, weighing approximately 120–140 g, were used. Rats were anaesthetized with Hypnorm (Duphar, Weesp, NL) containing fluanisone (10 mg ml^{-1}) and phentanylcitrate (0.2 mg ml^{-1}) in a dose of 0.08 ml kg^{-1} body weight. Crush lesioning of the peripheral nerve was performed as described in detail by De Koning *et al.* (1986). The right sciatic nerve was crushed for 30 s, with a haemostatic forceps. In one experiment male rats of different ages were used: rats of 6–7 weeks, 5 months and 20 months of age (weighing 150, 450 and 500–600 g, respectively) received a crush lesion for 30 s of the right tibial nerve. Hypophysectomy was performed under light anaesthesia by transauricular route as described previously (Gispen *et al.*, 1970). Five days after hypophysectomy ($n = 8$) or sham operation ($n = 16$), rats were either subjected to crush lesioning or to sham operation of the right sciatic nerve (see also legend to Figure 4). Reduction of body weight, adrenal atrophy to a wet weight of less than 10 mg and absence of tissue in the sella tursica upon postmortem macroscopic inspection, documented the efficacy of the surgery.

Functional recovery

The return of sensorimotor function was measured by the foot reflex withdrawal test, described previously (De Koning *et al.*,

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1986; Van der Zee *et al.*, 1988). The rat was immobilized by hand presenting the sole of its foot to the examiner. A small electric current was applied to the sole of the foot at a given place (position 3, see De Koning *et al.*, 1986) through two stimulation poles. A normal rat retracted its paw immediately when the skin of the foot sole closes the electric circuit. Rats bearing a crush lesion initially failed to do so. A response was scored as positive if the rat retracted its paw from the noxious stimulus instantaneously. A range of six brief pulses of current (0.1–0.6 mA) was used to ascertain the lowest strength of current required to elicit paw retraction. A rat was considered fully recovered (100% recovery) when it retracted its paw at a stimulus of 0.1 mA. Failure to retract at a 0.6 mA current stimulus indicated 0% recovery. Intermediate % recoveries were 17, 33, 50, 66 and 83 at 0.6, 0.5, 0.4, 0.3 and 0.2 mA respectively. The examiner performing the reflex withdrawal test was not aware of the treatment a given rat had received.

Data analysis

The treatment code was partially broken at the end of the experiment to allow analysis of the data obtained from the various treatment groups. The final treatment code indicating which group had received what treatment was broken only after analysis of the data was completed. The data obtained by the functional recovery test were expressed as the mean % recovery per day (±s.e.) and group differences were analyzed by an analysis of variance for repeated measurements (ANOVA), followed by a supplemental *t* test, using the raw data prior to transformation to percentage recovery.

Drugs

Peptides were synthesized and donated by Organon International B.V., Oss, The Netherlands. α-MSH (melanotropin), desacetyl-α-MSH, [N-Leu^4,D-Phe^7]-α-MSH, MSH$_{11-13}$, β-MSH, γ_2-MSH, ACTH$_{6-9}$, ACTH$_{7-16}$ and ORG 2766, an ACTH$_{4-9}$ analogue (H-Met(O$_2$)-Glu-His-Phe-D-Lys-Phe-OH). The peptides were freshly dissolved in saline and administered s.c. in a dose of 0.01, 0.05, 0.1, 1.0 or 10 μg 0.5 ml^{-1} 48 h^{-1}. The male rats of different ages and weights received ORG 2766 in a s.c. dose of 7.5 μg kg^{-1} 48 h^{-1}. Thyrotrophin-releasing hormone (TRH) was dissolved in saline and administered i.v. in a dose of 0.1 mg (bolus injection of 0.05 mg at 1 h and 3 h following surgery). Isaxonine (N-isopropyl-amino-2 pyrimidine orthophosphate, a gift from Organon International B.V.) was freshly dissolved in saline and administered i.p. daily in a dose of 35 mg. The ganglioside mixture (GA mixt = GM1, GD1a, GD1b, GT) was a gift from Fidia Research Laboratories (Abano Terme, Italy). The gangliosides were freshly dissolved in saline and administered i.p. in a dose of 0.7 mg per day. Nimodipine (a gift from Tropon GmbH, Köln, F.R.G.) was freshly dissolved in polyethyleneglycol (PEG) and administered i.p. daily in a dose of 20 mg kg^{-1} in 0.1 ml PEG per 100 g body weight. Control rats received vehicle s.c., i.v., i.p. or orally.

Results

Effect of putative neurotrophic compounds

In the first experiment different groups of rats, bearing a crush lesion in the right sciatic nerve, were treated with different putative neurotrophic compounds. The dose, route of administration and treatment schedule for each of the compounds was chosen from the literature and was reported to be optimally effective for the given drug (see legend to Figure 1). Routinely, rats treated with vehicle begin to respond to the noxious stimulus approximately at day 16–18 post operation and are considered fully recovered in this test at approximately 23–24 days (see control groups in Figure 1a,b,c,d).

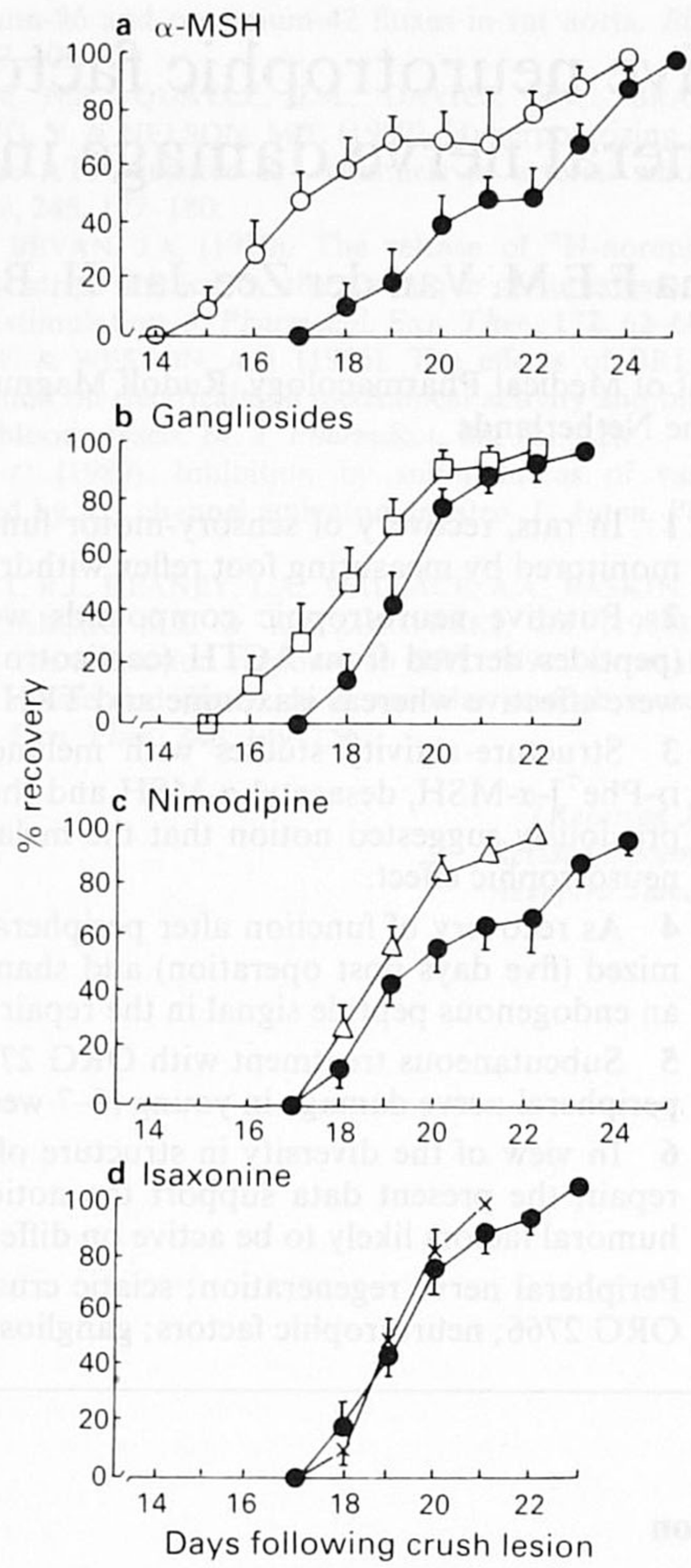

Figure 1 The effect of neurotrophic compounds on functional recovery following crush lesion of the sciatic nerve in the rat. The return of sensorimotor function was measured by the foot reflex withdrawal reaction after foot sole stimulation with a range of six current strengths of 0.1–0.6 mA. The data are expressed as the mean percentage (%) receovery per day (s.e.mean shown by vertical bars). In each group $n = 10$. (a) Rats receiving s.c. α-MSH (○), dose 10 μg 48 h^{-1}, showed a significantly enhanced recovery compared to rats receiving saline (●); $F(1, 18) = 14.5$, $P < 0.005$. (b) Treatment i.p. with gangliosides (□), 0.7 mg per day, significantly enhanced the functional recovery compared to saline treatment (●); $F(1, 18) = 4.7$, $P < 0.05$. (c) Nimodipine i.p., treatment (△), 20 mg kg^{-1} per day, resulted in a significantly enhanced recovery of function compared to treatment with the vehicle polyethylene glycol (●); $F(1, 18) = 5.6$, $P < 0.05$. (d) Isaxonine i.p., (x), 35 mg per day, showed no effect on the functional recovery following rat sciatic nerve crush, compared to saline (●). For the statistical evaluation an analysis of variance for repeated measurements (ANOVA) was performed using the raw data.

Treatment with α-MSH, gangliosides or nimodipine accelerated return of function substantially (Figure 1a,b,c), whereas treatment with isaxonine (Figure 1d) or TRH (data not shown) was ineffective.

Structure-activity studies with melanocortins

In order to gain insight into how the neurotrophic information is encoded within the α-MSH/ACTH molecule, various fragments and analogues were tested in the foot reflex withdrawal test as described above. Most peptides were tested at least in a dose of 1 or 10 μg per rat or both. In Figure 2 the '% recovery above control' at day 17 post surgery is presented. As can be seen, α-MSH, [N-Leu^4,D-Phe^7]-α-MSH, desacetyl-α-MSH and β-MSH were active whereas γ_2-MSH, containing the 4–9 sequence with one modification (Gly^5), was not. Interestingly, the short sequence MSH/ACTH$_{11-13}$ inhibited the

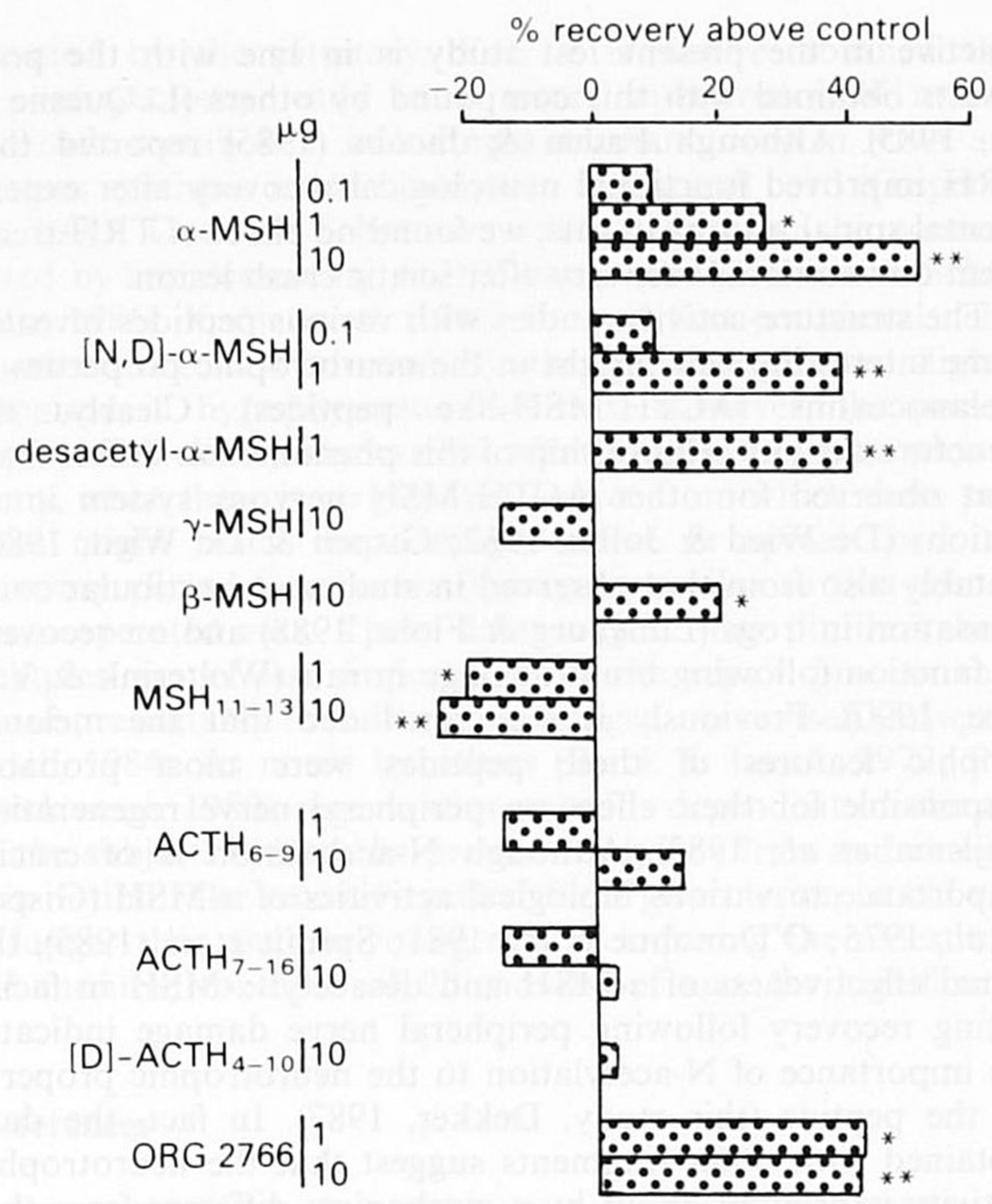

Figure 2 The effect of α-MSH/ACTH-fragments and -analogues on functional recovery following crush lesion of the rat sciatic nerve. The functional recovery was measured with the foot reflex withdrawal test (stimulus range 0.1–0.6 mA), and expressed as the mean percentage recovery. Each peptide-fragment or -analogue, in the dose(s) indicated in the Figure, was tested in a group of 10 rats and compared with its own saline-treated control group. The statistical evaluation (ANOVA) was performed on the raw data of all experimental days, comparing the peptide treatment values with the saline treatment values (as in Figure 1). In this diagram for each peptide-treated group ($n = 10$) the % recovery on day 17, with the % recovery of their own control group subtracted from it, is depicted as '% recovery above control'. Significantly enhanced functional recovery following crush lesion of the rat sciatic nerve is shown for α-MSH 1 μg ($F(1, 18) = 5.0$, $P < 0.05$) and 10 μg ($F(1,18) = 14.5$, $P < 0.005$); [NLeu^4,D-Phe^7]-α-MSH 1 μg ($F(1, 18) = 14.2$, $P < 0.005$); desacetyl-α-MSH 1 μg ($F(1, 18) = 13.4$, $P < 0.005$); β-MSH 10 μg ($F(1, 18) = 4.5$, $P < 0.05$); ORG 2766 1 μg ($F(1, 18) = 4.6$, $P < 0.05$) and 10 μg ($F(1, 18) = 11.1$, $P < 0.005$). The fragment MSH_{11-13} (doses 1 and 10 μg) inhibited significantly the functional recovery following crush lesion, $F(1, 18) = 4.5$, $P < 0.05$ and $F(1, 18) = 4.9$, $P < 0.05$, respectively. [N,D]-α-MSH = [N-Leu^4,D-Phe^7]-α-MSH; [D]-$ACTH_{4-10}$ = [D-Phe^7]-$ACTH_{4-10}$.
Significance: * $P < 0.05$; ** $P < 0.005$.

recovery of function, whereas $ACTH_{7-16}$ was without effect. The short sequence $ACTH_{6-9}$ and [D-Phe^7]-$ACTH_{4-10}$ were both inactive in this test paradigm whereas the $ACTH_{4-9}$ analogue ORG 2766 was active (Figure 2).

Neurotrophic activity of ORG 2766 at different ages

Male rats of 6–7 weeks, 5, 20 and 34 months were used. Crush lesioning of the right tibial nerve was performed on all rats at the same position. Rats of the first three age groups, were divided at random over two groups one of which was treated s.c. with ORG 2766 (7.5 μg kg^{-1} 0.5 ml^{-1} 48 h^{-1}) and the other treated with saline according to the same schedule. The oldest rats of 34 months of age ($n = 7$) received no treatment. As shown in Figure 3, control rats displayed return of sensorimotor function in an age-dependent manner. The younger the rat the earlier the return of sensorimotor function. The main effect of age between the saline group C and group D (oldest rats) was not significant. However, the interaction between age and time following surgery reached significance indicating a slower speed of recovery in group D (see Figure 3). In the three age groups treated with peptide (A,B,C), the neurotrophic influence of ORG 2766 on the recovery is evident, shown by a significantly enhanced functional recovery (see legend Figure 3).

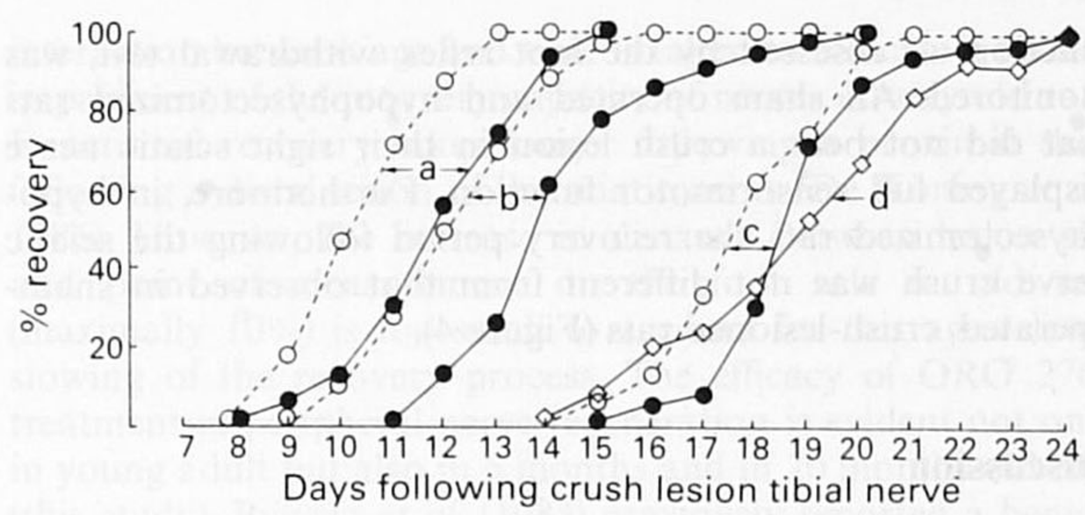

Figure 3 Effect of ORG 2766 administration following crush lesion of the tibial nerve in rats of different ages. The return of sensorimotor function following a crush lesion of the tibial nerve was measured with the foot reflex withdrawal test (stimulus range 0.1–0.6 mA), and expressed as the average % recovery (±s.e.mean < 20%, not shown). Rats were treated s.c. with ORG 2766 7.5 μg kg^{-1} 48 h^{-1} (○) or saline (●). (a) In 6–7 weeks old ORG 2766-treated rats ($n = 17$) the functional recovery was significantly enhanced compared to saline-treated age-matched rats ($n = 16$), $F(1, 31) = 4.4$, $P < 0.05$. (b) In 5 months old ORG 2766-treated rats ($n = 8$) a significantly enhanced functional recovery was apparent compared to saline-treated rats ($n = 8$) of the same age, $F(1, 14) = 20.2$, $P < 0.001$. (c) ORG 2766-treated rats of 20 months of age ($n = 5$) showed a significantly enhanced recovery of sensorimotor function compared to age-matched saline-treated rats ($n = 9$), $F(1, 12) = 4.7$, $P < 0.05$. In addition, both saline- and ORG 2766 peptide-treated groups showed an age-dependent regeneration rate; the young adult rats (a) reached 100% recovery earlier than the mature adults (b), and the latter regenerated faster than the old rats (c) ($P < 0.001$). (d) Non-treated rats of 34 months of age ($n = 7$) (□). Comparison between saline group (c) and (d): no main effect; interaction between age and time following operation $F(4, 56) = 2.7$, $P < 0.05$. For the statistical evaluation, an analysis of variance for repeated measurements (ANOVA) was performed using the raw data.

As a consequence of difference in bodyweight the distance between the distal border of the crush and the position of the footsole where the return of sensorimotor function was measured differed per control group. After completion of the experiments the exact measurements were taken and ranged from approximately 29 mm (6–7 weeks) to 32 mm (34 months).

Recovery of function following sciatic nerve crush in hypophysectomized rats

Five days after hypophysectomy or sham operation, rats were either subjected to crush lesioning or to sham surgery of the right sciatic nerve. Subsequently, the return of sensorimotor

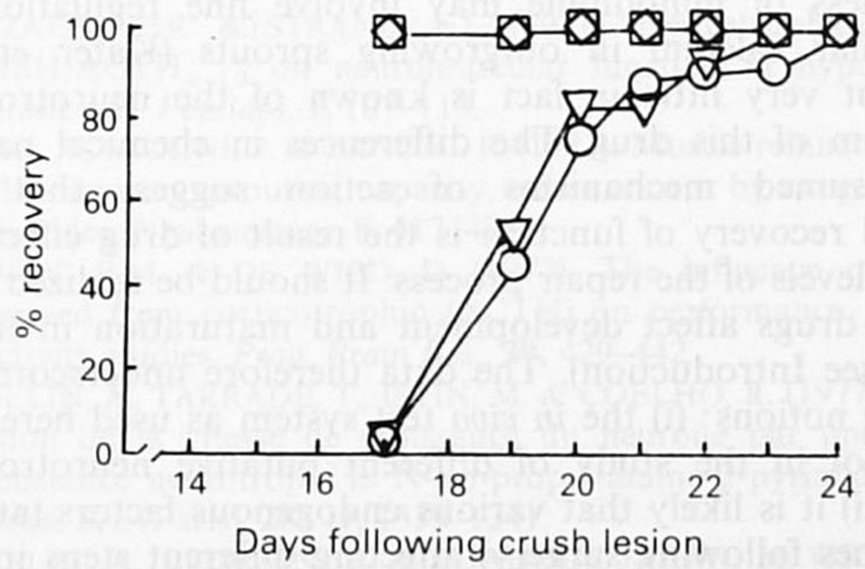

Figure 4 Recovery of function following sciatic nerve crush in hypophysectomized rats. Five days after hypophysectomy or sham operation, rats were subjected to sciatic crush lesion or sham lesion. The return of sensorimotor function was determined with the foot reflex withdrawal test (stimulus range 0.1–0.6 mA), and depicted as the mean percentage (%) recovery (± s.e.mean < 10%, not shown). The functional recovery following nerve crush in hypophysectomized rats (○, $n = 4$) was not different from sham-operated rats (▽, $n = 4$). Both sham-lesioned hypophysectomized (□, $n = 8$) and sham-operated (◇, $n = 8$) rats showed an intact sensorimotor function (reaction 100%).

function, as assessed by the foot reflex withdrawal test, was monitored. All sham operated and hypophysectomized rats that did not bear a crush lesion in their right sciatic nerve displayed full sensorimotor function. Furthermore, in hypophysectomized rats the recovery period following the sciatic nerve crush was not different from that observed in sham-operated, crush-lesioned rats (Figure 4).

Discussion

In the present study, we examined the efficacy of various putative neurotrophic compounds in a rat model of functional recovery following peripheral nerve damage. As shown previously in this model, the pattern of recovery over time is similar when responsiveness to a given stimulus rather than to a graded stimulus is measured or when the data are expressed as % recovery or as stimulus number of animals recovered (De Koning *et al.*, 1986; Van der Zee *et al.*, 1988). For the different treatment groups, the age and sex of the rats, the surgery and the test procedure were the same. However, the dose, route and schedule of treatment differed per drug and were identical to reported optimal conditions for the neurotrophic activity of the drugs (Hugelin *et al.*, 1979; Gorio *et al.*, 1983; Faden & Jacobs, 1985; Dekker *et al.*, 1987b; Van der Zee *et al.*, 1987; 1988). Indeed these optimal conditions resulted for α-MSH, gangliosides and nimodipine treatment in a facilitation of recovery of sensorimotor function following a crush lesion of the sciatic nerve. The precise mechanism of the neurotrophic action of these compounds is not yet fully understood. It should be kept in mind that neurotrophic activity as measured in an *in vivo* model might result from a direct neuronal effect or an indirect effect via non-neuronal elements of the nerve involved in the repair process.

Neurotrophic peptides of the ACTH/MSH family might exert their neurotrophic action by mimicking an endogenous signal early in the repair process originating from pro-opiomelanocortin (POMC) expression in the cell bodies or from neurofilament breakdown in the distal portion of damaged axons (for review see Bär *et al.*, 1990). Both melanocortins and gangliosides have been shown to improve neurite outgrowth into the distal nerve portion (Gorio *et al.*, 1983; Dekker, 1987; 1988), however, the peptides exclusively increase the number of newly formed sprouts not influencing their rate of outgrowth (Verhaagen *et al.*, 1987; Gerritsen van der Hoop *et al.*, 1988). Thus it is unlikely that an effect on denervation supersensitivity may account for the effect of melanocortins on peripheral nerve regeneration (see also Strand *et al.*, 1986). Ganglioside enhancement has been suggested to originate from a beneficial effect of NGF-receptor efficacy (Gorio *et al.*, 1983; Sebille, 1984; Ledeen, 1985). The effectiveness of nimodipine may involve fine regulation of intracellular calcium in outgrowing sprouts (Kater *et al.*, 1988), but very little in fact is known of the neurotrophic mechanism of this drug. The differences in chemical nature and presumed mechanisms of action suggest that the enhanced recovery of function is the result of drug effects at different levels of the repair process. It should be realized that all three drugs affect development and maturation in tissue culture (see Introduction). The data therefore underscore the following notions: (i) the *in vivo* test system as used here is a useful tool in the study of different putative neurotrophic agents; (ii) it is likely that various endogenous factors (at different times following surgery) affecting different steps in the repair process govern the recovery process; (iii) optimal recovery may be obtained by a therapy based on the combination of various neurotrophic agents (see also Varon, 1985). Development of new effective pharmacotherapy of peripheral nerve damage should take into account the consequences of these latter two notions.

The fact that isaxonine, although reported by some to be active in both development and repair of neurones (Hugelin *et al.*, 1977; 1979; Legrain, 1977; Sebille & Hugelin, 1982), was inactive in the present test study is in line with the poor results obtained with this compound by others (LeQuesne *et al.*, 1985). Although Faden & Jacobs (1985) reported that TRH improved functional neurological recovery after experimental spinal trauma in cats, we found no effect of TRH treatment on functional recovery after sciatic crush lesion.

The structure-activity studies with various peptides revealed some interesting new insight in the neurotrophic properties of melanocortins (ACTH/MSH-like peptides). Clearly, the structure-activity relationship of this phenomenon differs from that observed for other ACTH/MSH nervous system interactions (De Wied & Jolles, 1982; Gispen & De Wied, 1984), notably also from that observed in studies on vestibular compensation in frogs (Lüneburg & Flohr, 1988) and on recovery of function following brain damage in rats (Wolterink & Van Ree, 1990). Previously it was concluded that the melanotrophic features of these peptides were most probably responsible for their effect on peripheral nerve regeneration (Bijlsma *et al.*, 1983). Although N-acetylation is of crucial importance to various biological activities of α-MSH (Gispen *et al.*, 1975; O'Donohue *et al.*, 1981; Spruijt *et al.*, 1985), the equal effectiveness of α-MSH and desacetyl-α-MSH in facilitating recovery following peripheral nerve damage indicates no importance of N-acetylation to the neurotrophic property of the peptide (this study, Dekker, 1987). In fact, the data obtained with other fragments suggest that the neurotrophic activity is brought about by a mechanism different from that of the classical melanotrophic activity. For, the potentiated MSH peptide [N-Leu^4,D-Phe^7]-α-MSH, extremely potent in the melanophore assay (Sawyer *et al.*, 1980) and in the excessive grooming test (Spruijt *et al.*, 1985), is as effective as the parent molecule α-MSH in enhancing peripheral nerve regeneration. β-MSH containing the same 4–10 sequence is also active, but γ_2-MSH with one substitution (Gly^5) in the 4–9 sequence is inactive. This latter peptide was also inactive in facilitating functional recovery in rats with bilateral parafascicular lesions (Nyakas *et al.*, 1985) and in vestibular compensation rate after labyrinthectomy in frogs (Lüneburg & Flohr, 1988). The sequence (11–13) which is known to represent a dormant information site additional to the region (4–10) (Eberle & Schwyzer, 1975; Schwyzer, 1980; Eberle *et al.*, 1985), was shown to inhibit rather than to stimulate the recovery of function following peripheral nerve damage (Figure 2). To our knowlege the only other reported inhibition by neuropeptides is the observation by the group of Strand which demonstrated that the deleterious changes in neuromuscular function (muscle action potential and muscle contraction) in hypophysectomized rats were exacerbated by high doses of $ACTH_{4-10}$, although low doses had the opposite effect (Gonzalez & Strand, 1981); subsequently they observed in 14-day-old peptide-treated rats, that high but not low doses of ORG 2766 lead to impoverished rather than enriched nerve terminal branching during maturation of the motor endplates (Frischer & Strand, 1988). Finally, the ACTH4–9 analogue, ORG 2766, originally developed to diminish melanotrophic activity and to be more stable than the parent sequence (Greven & De Wied, 1973), was as active as α-MSH. Thus at present it remains uncertain what the precise localization of the neurotrophic activity is, although the region 4–10 is critical. As observed previously (Bijlsma *et al.*, 1983), changes in the region 4–10, the ultimate message region for the biological activity of ACTH/MSH (Schwyzer, 1980; Eberle *et al.*, 1985) often affect the neurotrophic activity of these peptides. Thus γ_2-MSH, $ACTH_{6-9}$, $ACTH_{7-16}$ and [D-Phe^7]- $ACTH_{4-10}$ with an incomplete or modified 4–10 sequence are ineffective, whereas in contrast $ACTH_{6-10}$ and the modified 4–9 analogue ORG 2766 are effective (see also Bijlsma *et al.*, 1982; De Koning *et al.*, 1986; Dekker, 1987; Van der Zee *et al.*, 1988).

In attempting to understand why exogenous administered fragments or analogues of endogenous circulating peptide hormones facilitate the repair of damaged peripheral nerve, the question arises whether these fragments mimic peptides

secreted by the pituitary. For, it is well established that hypophysectomy leads to a severe interference of macromolecule metabolism including a dramatic drop in RNA and protein synthesis in rat brain stem and spinal cord (Gispen *et al.*, 1970; Dunn & Schotman, 1986), which can be counteracted by supplementing ACTH-like peptides (Dunn & Schotman, 1986). Kanje *et al.* (1988) showed that peripheral nerve regeneration, as evaluated by the 'pinch-test', was slightly impaired in hypophysectomized rats. However, the present experiment clearly shows that after five days of hypophysectomy, when there is no pituitary source from which these peptides might reach the damaged nerve or spinal cord, the recovery period from nerve damage is similar to that seen in sham-operated rats. These data suggest that pituitary born peptides are of less importance to the repair process than those presumably found in the damaged nerve itself (Edwards *et al.*, 1984). As noted by others (Black & Lasek, 1979; Pestronk *et al.*, 1980), regeneration speed is dependent on the age of the subject. Indeed the present study confirms and extends this notion. The recovery period for a given lesion in old rats is longer than that in young rats. Comparing the oldest rats (34 months) to those of 20 months of age, the significance interaction between age and time is taken to indicate a further impairment of the recovery capacity of nerves of very old rats. Functional recovery takes longer following a proximal than following a distal lesion of the sciatic nerve (De Koning *et al.*, 1986). However, the increase in distance between lesion site and point of measurement on the foot sole in old rats (maximally 10%) is too small to account for this age-related slowing of the recovery process. The efficacy of ORG 2766 treatment in peripheral nerve regeneration is evident not only in young adult but also in 5 months and in 20 months old rats (this study). Bijlsma *et al.* (1983) previously reported a beneficial, though small, peptide effect in one year old rats.

In conclusion, the present study demonstrates that neurotrophic factors of different nature and mechanism of action may facilitate the recovery of function of damaged peripheral nerve *in vivo*. Furthermore, the data indicate that ACTH/MSH-like peptides are effective in young adult, mature and old rats. If they are effective by mimicking an endogenous peptide signal, that peptide is not of pituitary origin. Collectively the structure activity studies on the neurotrophic activity of ACTH/MSH-like peptides do question the conclusion that melanotrophic activity is responsible for the effect.

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