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NEUROTROPHIC EFFECTS OF ACTH/MSH NEUROPEPTIDES

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Abstract. Numerous experiments with peptides related to ACTH/MSH, and involving tests such as avoidance, approach, discrimination and rewarded behavior indicate that these peptides possess neuroactive effects on learning, motivation, attention, and concentration. In addition, ACTH/MSH neuropeptides affect social behavior, interact with opiate binding sites, and possess antiepileptic properties. Other CNS effects which can be demonstrated after intracranial administration only are grooming behavior, stretching, yawning and sexual behavior. The effects reside mainly in the N-terminal part of ACTH (ACTH-(4-10); ACTH-(7-16) and are dissociated from the peripheral corticotrophic effect. Several substitutions in the sequence ACTH-(4-9) led to a highly selective, potent and orally active neuropeptide with a marked loss of endocrine effects. Thus H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766) appeared to be 1,000 times more active on avoidance behavior than ACTH-(4-10) but to contain 1,000 times less melanotrophic activity. It also had a markedly reduced steroidogenic, fat mobilizing and opiate-like activity. ACTH/MSH peptides also possess neurotrophic activities as derived from studies on regeneration of damaged nerve cells. Animal studies show beneficial effects of semichronic treatment of the ACTH-(4-9) analogue Org 2766 on nerve crush regeneration in animals. The activity for this effect resides in the sequence ACTH-(6-10). The neurotrophic influence is evident both at the sensory and the motor function level. The protective effect of Org 2766 is also found in other neuropathies as a result of diabetes mellitus and chemotherapy. It has been po-

stulated that ACTH peptides mimic a signal as part of a regeneration program of the neurons similar to that found in developing nerves. Also recovery from lesions in the nucleus parafascicularis, nucleus accumbens and fimbria fornix can be facilitated by treatment with ACTH neuropeptides. In addition, long-term treatment of rats with Org 2766 reduces morphological correlates of brain aging such as neuronal loss and increased glial reactivity, neurochemical correlates such as corticosterone receptor loss and functional correlates such as emotionality, cognitive function and sociability.

Numerous experiments with peptides related to ACTH/MSH, and involving tests such as avoidance, approach, discrimination and rewarded behavior indicate that these peptides affect learning, motivation, attention, concentration and memory processes. In addition, ACTH/MSH neuropeptides affect social behavior, interact with opiate binding sites, and possess anti-epileptic properties. Other CNS effects which are only demonstrated after intracranial administration are grooming behavior, stretching, yawning and sexual behavior. Besides these neuroactive effects, ACTH/MSH neuropeptides possess neurotrophic influences (59).

Initially a great number of experiments was performed to determine the active locus in the ACTH molecule for the effect on avoidance behavior. These structure activity studies were performed for the greater part on extinction of pole jumping avoidance behavior in intact rats. In this test ACTH-(1-24), ACTH-(1-10), ACTH-(4-10) and ACTH-(4-7) were equally active. Removal of the amino acid residues 4 or 7 markedly reduced the behavioral effect (23). Peptides as ACTH-(7-10), Ac-ACTH-(11-13)NH_2 and ACTH-(11-24) had some residual activity. The residual potency of ACTH-(7-10) could be increased to the same level as ACTH-(4-10) extending the C-terminal sequence to ACTH-(7-16)NH_2. Thus, other sequences such as (11-13) contain elements for behavioral activity which are present in a latent form. Substitution of phenylalanine by a D-enantiomer in ACTH-(1-10), ACTH-(4-10) or ACTH-(4-7) had an effect opposite to that of the L-enantiomer peptides. These D-Phe^7 analogs facilitate extinction of shuttle box and pole-jumping avoidance behavior (23). Several substitutions in the sequence ACTH-(4-9) led to a highly selective and potent neuropeptide with a marked loss of endocrine effects. Thus, H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766) appeared to be 1,000 times more active than ACTH-(4-10) but to contain 1,000 times less melanotrophic activity. It also had a markedly reduced steroidogenic, fatmobilizing and opiate-like activity. Because of its increased resistance to enzymatic degradation Org 2766 also appeared to be orally active (24). The substitutions cause not only potentiation and

prolongation of the action but also the incorporation of other intrinsic activities. Org 2766 elicits opposite effects in that "low" amounts facilitate while "high" doses attenuate passive avoidance behavior and electrical brain self-stimulation. The effects of "high" doses can be blocked by the opiate antagonist naltrexone and appear to reside in the C-terminal tripeptide Phe-D-Lys-Phe (PDLP) (16, 17).

Postlesion neuronal repair is often viewed as a rapid replay of those cellular processes that govern neuronal development, maturation and network formation. Thus humoral factors that influence neuronal development may be of benefit to neuronal repair mechanisms in adult life. Recently, several authors have shown that ACTH/MSH-like peptides influence the growth and differentiation of central neurons in culture. Daval et al. (8) treated cerebral chicken neurons with ACTH-(1-24) and observed an enhancement of cell metabolism in general, and increased neurite formation. The effect appeared to be dose-dependent, with a maximum at 10 nM ACTH-(1-24). Azmitia and De Kloet (1) used cultures of dissociated rat hippocampal and raphe cells. They showed an enhancement of serotonergic maturation by various ACTH-like peptides (ACTH-(1-24), ACTH-(4-10) and Org 2766, provided that the raphe cells were cultured alone. In the presence of the natural hippocampal target cells of these raphe cells no effect of the peptides was observed, suggesting that they may exert their neurotrophic influence on raphe cells via similar mechanisms as hippocampal target cells through a soluble protein that normally stimulates the outgrowth of raphe cells (1). Richter-Landsberg et al. (44) studied the effect of ACTH-(4-10) and ACTH-(1-24) on the growth and development of embryonic rat cerebral cells in culture. Long-term peptide treatment resulted in an increase in the density of the neuronal network, in neuronal aggregates and neurite bundles. At the same time a 30% increase in acetylcholinesterase (AChE) activity was observed. Van der Neut et al. (39) used slices of foetal rat spinal cord in culture to establish neurotrophic effects of α-MSH and ACTH-(4-10). After 5 and 7 days, outgrowth was quantified with two different techniques, namely a visual scoring under phase-contrast and an ELISA for neurofilament protein. Both peptides dose dependently stimulated the formation of neurites by 30-40% in the sub-nanomolar range.

Earlier studies had already pointed to neurotrophic effects of melanocortins on neural development *in vivo*. In a series of experiments Swaab and coworkers obtained evidence that α-MSH but not other ACTH related peptides or Org 2766 affects intrauterine foetal rat body and brain growth. Interestingly, the trophic effect of α-MSH was not detected when the peptide was given in the first weeks of postnatal life

(see for review 54). Van der Helm-Hylkema and De Wied (26) reported a stimulatory effect of ACTH and fragments (ACTH-(1-39), (1-24), -(1-18), -(1-16) on eye opening of both male and female rat pups. The shorter peptides α-MSH, ACTH-(1-10), ACTH-(4-10) or Org 2766 were not effective. How ACTH affects eye-opening is not clear, although it is tempting to assume that it is brought about by a direct effect on the developing rat central nervous system. In another series of experiments Strand and coworkers reported a beneficial effect of ACTH-(4-10) and Org 2766 on perinatal development of rat motor function and neuromuscular junction. Their neurophysiological data suggested an improvement though a neurotrophic effect of the peptide, although the effect on endplate maturation suggested a myotrophic action as well (see for deview 52).

ACTH-like peptides exert a beneficial role in the repair and development of the nervous system (see for review 51). Therefore, these peptides can be considered as small growth factors with a completely specified primary structure. Evidence for neuroptrophic effects on the peripheral nervous system is well documented. ACTH/MSH peptides facilitate the recovery following a sciatic nerve crush in the rat (3, 31, 50). The most active peptides in this respect are α-MSH and Org 2766, the shortest active fragment appeared to be ACTH-(6-10) (2, 5, 9). These findings led to the suggestion that melanotrophic rather than corticotropic features of the ACTH/MSH peptides are involved. However, ACTH-(6-9) which is essential for melanotrophic activity by itself is not effective (Van der Zee, personal communication). The neurotrophic influence of ACTH/MSH-like peptides was evident both at the sensory (19, 31) and the motor function level (32). The latter was apparent in improvement of the walking pattern following peptide treatment of rats bearing unilateral crush lesions in their sciatic nerve. Studies on the optimal route of administration revealed neurotrophic activity following subcutaneous injection and topical application of the peptide at the site of the lesion. Oral administration was shown to be ineffective (9, 11, 64). In addition, the treatment should commence immediately following the damage, in other words there is strong evidence for a critical effective treatment period (13, 55, 57). These functional data are corroborated by neurophysiological and histological evidence. It was shown that peptide treatment of rats bearing a sciatic nerve crush lesion improves several parameters of muscle function such as muscle action potential motor unit activity etc. and nerve muscle efficacy (46). Furthermore, it was found that treatment resulted in the formation of small motor units, allowing a more finely-graded control of muscle contraction (47). Finally, it was demonstrated that short-term peptide treatment led to long-term beneficial effects on motor nerve conduction and H-related sensory-nerve

conduction velocities in the regenerating sciatic nerve (32). Based on histological analysis of the crushed nerve at first it was proposed that the neurotrophic influence was based on enhanced outgrowth of newly formed sprouts (50). However, later work revealed unequivocally an increase in the number of sprouts branching from the proximal nerve stump (2, 4, 5, 55) which is more in line with the functional data at hand (19). Recently it was observed that peptide treatment also enhanced collateral sprouting following partial denervation of the soleus muscle in the rat (34). This is an interesting observation as the process of collateral sprouting is presumably under different control than sprouting. Such activity may be of importance in CNS post-lesion plasticity.

To obtain more insight in whether the enhanced return of function is caused by an action of the peptide on the speed of outgrowth or on the actual number of newly formed sprouts, a crush model of the tail nerves of the rat was developed. By recording the time needed for return of sensory function on various points of the tail, each located more distally with respect to the crush site, it was established that Org 2766 treatment enhanced the return of sensory function with about 2-3 days, but the subsequent rate of recovery was approximately the same in both peptide and saline treated groups (20). Interestingly, melanocortins also promote collateral sprouting: experiments in which the rat soleus muscle was partly denervated by cutting tho lumbar L5 root, showed collateral sprouting from the remaining motor units innervated by L4 and L6 roots. In saline treated rats these motor units could increase their muscle fibers five-fold, whereas in peptide treated animals the increase was about 7.5-fold (34).

Several observations suggest that melanocortins mimic or amplify a natural occurring peptide signal early in the repair process. Transected peripheral nerves excrete neurotrophic proteins which facilitate nerve fibre regeneration (42). These are probably taken up by the axons and transported to the cell bodies (48). Currently, two working hypotheses are being considered. One hypothesis suggests that the distal degenerating nerve segment produces an MSH-like peptide that faciliates sprouting from the proximal stump (10, 13). The source of the peptide could be the neurofilament protein NF 150 (13, 58). Although the distal degenerating nerve seems to produce MSH-like activity as determined by bio-assay (13), the factor has not yet been characterized (56). Another observation by Lunn et al. (38) in a mutated mouse strain questions the need of degenerative products. They found in mice, in which leucocyte invasion is slow and sparse and the myelin removal extremely slow, that the absence of Wallerian degeneration does not hinder the regeneration in peripheral nerve. The alternative or additional possibility

is that the cell body of damaged peripheral nerves reexpress proopiomelanocortin (POMC) (25, 27). Evidence is accumulating that embryonic motor neurons express POMC (7). In the mature peripheral nervous system this does not occur, except in genetically dystrophic mice in which POMC expression is related to the growth and maturation repertoire of damaged neurons. Indeed, preliminary results indicate that POMC expression, measured by in situ hybridization with an Exon 3 probe, is significantly increased after a sciatic crush lesion, both in dorsal root ganglia and spinal cord (Plantinga, Lopes da Silva, Schrama, personal communication). Although further experimental support is needed to clarify the mechanism of action of neurotrophic peptides, the realization that the formation of a naturally occurring signal might be an intrinsic property of the damaged nerve, offers a scala of important clinical applications. Based on the hypothesis that exogenous administered peptides might mimic or amplify an endogenous signal that triggers the regenerative response, it was postulated that not only mechanical damage but also other neural damage, like neurotoxic-induced or diabetic-induced peripheral neuropathy could benefit from peptide treatment. Examples of cumulative toxins which induce peripheral neuropathy are acrylamide and the antitumor drug cisplatin. Acrylamide causes a mainly peripheral neuropathy in man and animal. The toxin results in a Wallerian degeneration of distal regions of nerve axons and many of the biochemical changes that occur resemble those observed following axotomy (6). The eventual recovery is the result of regeneration of the surviving proximal parts of the nerve (53). Rats treated with acrylamide (50 mg/kg, every 48 h) for two weeks develop severe abnormalities in the walking pattern and the landing foot spread. Also the sensory nerve conduction velocity (SNCV) is diminished. Concomitant treatment with Org 2766 does not affect the onset of the neuropathy. However while saline treated animals still display a decreased SNCV 88 days after the beginning of the treatment with acrylamide, complete normalization occurs in peptide-treated animals (12). The anti-tumor drug cisplatin, used in the chemotherapy of ovarian and testicular cancer patients, causes a purely sensory neuropathy at cumulative dose levels (41). Rats given cisplatin (1 mg/kg) twice a week develop a sensory neuropathy after 7 weeks of treatment as indicated by a marked decrease in SNCV (33). Cotreatment with Org 2766 protects cisplatin-induced sensory neuropathy and also improves the SNCV when the neuropathy is already manifest, even during continuing cisplatin administration (19, 33). Morphological analysis of sural nerves of rats that were given cisplatin for 12 weeks revealed no change in the total number of fibers, but a decrease in the number of thick myelinated nerve fibers and a reduced

degree of myelination. Animals that received Org 2766 treatment showed a normal distribution of sensory nerve fibers (21).

Peripheral neuropathy also occurs in diabetes mellitus. It is characterized by greatly decreased peripheral motor nerve and sensory nerve conduction velocities. Also autonomic nervous system neuropathy occurs in this disorder which affects particular the gastrointestinal, cardiovascular and urogenital system (15). In streptozocin-induced diabetes mellitus in rats a delayed sensory nerve conduction velocity (SNCV) is found approximately after 9 weeks. Treatment with Org 2766 restored SNCV to normal values, not significantly different from nondiabetic controls. In saline treated diabetic rats an impaired pressor response to phenylephrine and tyramine is found, indicative of an impaired autonomic nervous system function of the circulation. Org 2766 restored the tyramine responsiveness, whereas the impaired phenylephrine response was not affected. Org 2766 peptide treatment apparently protects from streptozocin-induced diabetic peripheral neuropathy.

ACTH/MSH neuropeptides exert neurotrophic effects both in animals and in humans. A multi-center, placebo controlled clinical trial in 55 ovarian cancer patients treated with cisplatin was set up to study the possible preventive action of Org 2766 in human subjects. In this study 2 doses of Org 2766 (0.25 mg/m^2 and 1 mg/m^2) were compared to placebo treatment at the start, and after 2, 4 and 6 cycles of cytotoxic treatment. The vibration sense was used as the main parameter, since earlier studies indicated that this parameter was affected in a subclinical phase of the neuropathy (14, 45). Vibration perception thresholds began to increase, implicating a gradual loss of function, in the placebo group after 2 treatment cycles and further deteriorated after 4 and 6 cycles. In the high dose Org 2766 treatment group no change was seen after 2 and 4 cycles, whereas only a modest increase was seen after 6 cycles. The differences were statistically highly significant. The low dose peptide group displayed a moderate loss of vibration sense. Symptoms (paraesthesia, numbness) and sings (loss of tendon reflexes, vibration sense and fine touch perception) occurred in 50% of the patients in the placebo group after 4 courses, but were not seen in the high or low dose Org 2766 treatment groups. There existed still a substantial difference compared to the placebo group after 6 cycles. The response to cisplatin treatment was similar in all three groups. These results suggest that Org 2766 is capable of preventing or at least postponing cisplatin neuropathy in ovarian cancer patients (22).

Several reports have been published which suggest that damage in the central nervous system may be reduced by treatment with melanocortins. Flohr and Lüneburg (18) observed that systemic treatment with

ACTH-(4-10) improved the acquisition and maintenance of the compensated state which follows unilateral labyrinthectomy in *Rana temporaria*. Lüneburg and Flohr (37) found similar effects with α-MSH, the synthetic ACTH analogs Org 2766 (4-9) and Org 5041 (4-16), ACTH-(4-7), ACTH-(1-10) and ACTH-(4-10). Likewise, Igarashi et al. (28, 29) reported that ACTH-(4-10) modified the characteristics and time courses of vestibulo-spinal and vestibulo-oculomotor balance compensation following labyrinthectomy in the squirrel monkey. ACTH-(4-10) appeared to contribute to the organic repair or remodelling of the neural network related to the motor output system (28).

ACTH/MSH peptides might also affect the diminished neural plasticity in the CNS during aging (18*a*). At the behavioral level aging is characterized by behavioral deficits of which cognitive dysfunction and reduced sociability are well documented expressions. Long-term treatment with Org 2766 reduces the morphological and functional correlates of brain aging such as neuronal loss, increased glial reactivity and a reduced maze reversal learning (36). In a subsequent study Landfield (35) showed that Org 2766 inhibited age-related neuronal or synaptic loss and reduced multiple synaptic contacts which may be a compensatory response to synaptic loss. Long-term treatment of old rats with Org 2766 reverses the loss of corticosterone type 1 receptor in senescent rats (43). Spruijt et al. (49) showed that treatment of 19 months old rats for 6 months delayed signs of aging significantly faster in learning a watermaze and also showed more social behavior. This was still found two months after discontinuing the treatment and suggests the lasting nature of the effect of Org 2766. Brain damage might also benefit from treatment with ACTH/MSH neuropeptides. Org 2766 facilitates recovery from hyperemotionality in rats with lesions in the septal area (30). The same compound and α-MSH facilitate recovery of a reversal learning impairment following lesions in the nucleus parafascicularis of the thalamus (40). Such lesions impaired a T-maze reversal learning, disturbed motor function and increased the grasping response. Org 2766 treatment or treatment with α-MSH but not with γ_2-MSH induced recovery of the cognitive impairment but not of other behavioral disturbances of lesioned rats. Treatments were effective when given either during the first or during the second week after the lesion but acute treatment was without effect. These studies did not search for morphological and biochemical correlates of the recovery and were performed in rats bearing electrolytic lesions in the brain. The results in rats with electrolytic lesions suggested that ACTH/MSH peptides might be also beneficial in animals in which central nervous function was eliminated by local injections of 6-OHDA. Chronic treatment either into the nucleus accum-

bens or subcutaneously with Org 2766 shortened recovery of bilateral 6-OHDA lesion of the nucleus accumbens from 28 days to 7 days (62). The treatment does not affect lesion induced changes in accumbal DA, DOPAC and HVA. Scatchart plot of ^{3}H-haloperidol again showed two binding sites and behavioral supersensitivity of apomorphine. Thus Org 2766 accelerates functional recovery by affecting processes involved in spontaneous recovery. Functional recovery of impaired motor activity caused by bilateral 6-OHDA lesions of the nucleus accumbens could also be accelerated by other ACTH/MSH neuropeptides (60). A structure activity study showed that ACTH-(4-10), α-MSH, ACTH-(7-10) were as effective as Org 2766. However, ACTH-(4-7) which is the active core for cognitive behavior (59) is inactive as is the C-terminal part of Org 2766 Phe-D-Lys-Phe (PDLP). It could be that the neurotrophic site is present in the 7-10 portion of the ACTH-molecule but this does not agree with the failure of PDLP to cause a similar beneficial effect. Finally Wolterink et al. (63) were able to show that α-MSH or Org 2766 antiserum in the nucleus accumbens of a 6-OHDA lesioned rat delayed functional recovery. This suggests that endogenous ACTH/MSH factors mediate recovery processes in the brain or are needed to maintain an optimal function of nervous system cells. The delay in recovery is accompanied by the absence of apomorphine hypersensitivity as seen in 6-OHDA-lesioned rats at 3 weeks after the lesion and of the two-type affinity model for ^{3}H-haloperidol binding sites. It thus looks as if the antisera blocked postsynaptic supersensitivity. Greven and De Wied (23) have shown that Org 2766 is orally active and found that one needs orally 1,000 times more than subcutaneous administration as measured on extinction of pole-jumping avoidance behavior. I.c.v. injection elicited a similar effect again in a dose 1,000 times less than needed subcutaneously. The doses used by Wolterink and Van Ree (61) were 80.6 μg/kg for oral and 28.5 ng/kg for subcutaneously and only 0.76 ng/kg for intraaccumbal administration. In this study they also showed that treatment during the first 6 days after the lesion is necessary to facilitate functional recovery. Treatment during days 1-3 or 4-6 or a single injection at day 6 was inneffective.

The above mentioned experiments show that ACTH/MSH-like neuropeptides possess neurotrophic activities. Beneficial effects of these neuropeptides have been found on regeneration of damaged central and peripheral nerve tissue as well as on aging following semichronic and chronic treatment. These peptides may also be clinically effective and may have important potential in preventing peripheral neuropathies, in facilitating recovery of brain lesions and in delaying symptoms of aging.

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