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David and Goliath — the slingshot that started the neuropeptide revolution

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Abstract

This review in honor of David de Wied summarizes the work done in my laboratory that first indicated that adrenocorticotropic hormone (ACTH) has a direct effect on the neuromuscular system. Cold stress or ACTH and its related peptides α-melanocyte-stimulating hormone (α-MSH) and β-lipotropin improve the electromechanical characteristics of adrenalectomized and hypophysectomized rats. ACTH-(1–39) accelerates the return of motor and sensory function and improves the morphological characteristics of the motor endplate after peripheral nerve crush. The non-corticotropic fragments ACTH-(4–10), α-MSH, the ACTH-(4–9) analogue Organon 2766 (Org 2766) or the ACTH-(4–10) analogue Biomeasure 22015 (BIM 22015) improve electrophysiological and morphological parameters of the regenerating neuromuscular system. ACTH-(4–10) immunoreactivity, present in ventral horn motor neurons in low levels, is decreased ipsilaterally following ipsilateral nerve crush but increases both ipsilaterally and contralaterally if injured animals are treated with ACTH-(4–10) indicating a neuroprotective action. Similarly, Org 2766 appears to have a protective action in the brain following nigrostriatal lesions. In developmental studies, perinatal exposure to ACTH peptides improves the structure of the neuromuscular junction, accelerates the maturation of electromechanical properties and enhances nerve–muscle integration and nerve regeneration. Perinatal exposure to these peptides decreases adult male sexual behavior, a change correlated with increased serotinergic input within the medial preoptic area. Similar changes occur in female rats and appear to be long-lasting. In tissue culture studies, both Org 2766 and BIM 22015 promote neurite outgrowth in the absence of nerve growth factor, indicating a neurotrophic role for these peptides.

Keywords: Regeneration; Development; Melanocortin

1. Introduction

I first met David de Wied in Munich in 1961, at a meeting of the International Physiological Society. This was a turning point in my scientific career and a joyous occasion for I made a new friend. I had been doing research on the effects of cold stress on the neuromuscular system of the rat and I, together with my colleagues in Berlin, had found that muscle action potentials were increased following cold stress in both intact and adrenalectomized animals, from which we inferred that endogenous adrenocorticotropic hormone (ACTH) could act directly on nerve and muscle. This heresy resulted in the rejection of our manuscript by several reputable journals. As it had not occurred to me to follow the literature on the behavioral effects of ACTH, I was naively unaware of the pioneering De Wied's work on the direct effects of ACTH on the behavior of hypophysectomized rats (De Wied, 1969). He encouraged me to repeat some of my experiments on hypophysectomized animals and resubmit the manuscript, citing his results in support of the concept of an extra-adrenal effect of ACTH. This paper was finally published (Strand et al., l973/1974) and so commenced a series of studies which branched out in many directions, each answer initiating more questions, making my scientific life exciting, rewarding and sometimes frustrating.

It was in these early days that De Wied coined the term "neuropeptide" (De Wied, 1969) a definition that has been extended today to include not only those peptides of neural origin but also those of non-neural origin that profoundly affect neural functions (De Wied, 1999). Thus, the neuropeptide concept, brilliantly described by De Wied (1987) in detail, has become ever more important and encompass-

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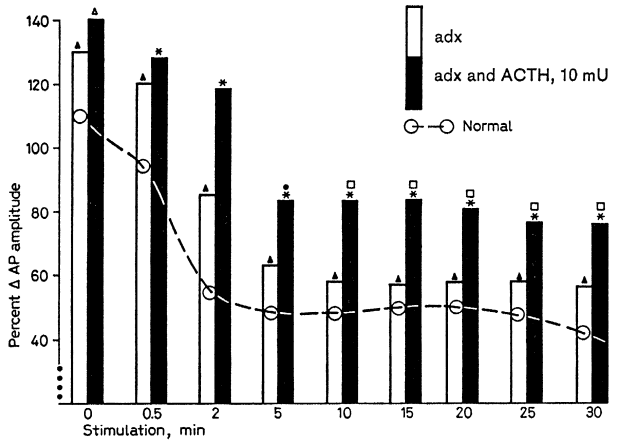

Fig. 1. Increase in action potential (AP) amplitude and decrease in fatigue following adrenalectomy (adx) and ACTH administration. *: $P < 0.01$ vs. normal; □: $P < 0.01$ vs. adx; ▲: $P < 0.05$ vs. normal; ●: $P < 0.05$ vs. adx. From Strand et al. (1973/1974).

ing. It is amusing that a controversy over this definition has recently been raised by way of a review (Iverson, 1999; De Wied et al., 2000; Kastin, 2000) of my book (Strand, 1999). David is still battling Goliath but I think he has slain the giant. It took years to gain acceptance for the concept of passage of peptides through the blood–brain barrier (Banks and Kastin, 1985), a vital link for the acknowledgement that peripherally administered melanocortins (non-corticotropic fragments of ACTH and their analogues) could affect central neurons. And it took decades to accept that peripherally administered melanocortins could accelerate nerve regeneration, improve neuromuscular performance and drastically affect the development of fetuses and neonates. In addition to these effects, and those on learning and behavior, the melanocortins have a direct effect on many other physiological functions, including weight homeostasis, anti-inflammatory and antipyretic actions and interaction with opiates and monoamines. However, even the most reluctant scientists have eventually accepted these concepts and forgotten their initial criticisms. The cloning and isolation of specific melanocortin receptors in both the central nervous system and in peripheral organs has lent scientific credence to the many diverse actions of the melanocortins (see review by Adan and Gispen, 1997).

2. Studies on adrenalectomized and hypophysectomized animals

As an historical review, I would like to trace the progress of the studies in my laboratory on the direct effects of the melanocortins on neuromuscular parameters, starting with experiments that showed that the closely related peptides, ACTH, α-melanocyte-stimulating hormone (α-MSH) and β-lipotropin (β-LPH) all exert modulatory effects on neuromuscular function (Strand and Cayer, 1975; Strand et al., 1976). This family of peptides, which derive from the large pituitary peptide pro-opiomelanocortin, regulate the excitability of neurons on many levels of the nervous system, including the reticular formation, the spinal cord and the locus ceruleus, important components of motor pathways. Specifically, administration of these peptides increases the amplitude of nerve and muscle action potentials, and decreases fatigue in adrenalectomized (Fig. 1) and hypophysectomized rats (Gonzalez and Strand, 1981). This facilitatory response can be evoked by non-corticotropic fragments of ACTH, such as ACTH-(4–10) and the ACTH-(4–9) analog, Organon 2766 (Org 2766). α-MSH, which has the amino acid sequence of ACTH 1–13, exerts essentially similar neurogenic influences. These peptides have little or no effect on the normal, rested neuromuscular preparation. However, these pro-opiomelanocortin-derived peptides are effective in animals with disturbed metabolism, such as diabetes, genetic obesity, or motoneuron disease (Hughes and Smith, 1989; Hughes et al., 1992). This concept has been further extended to several clinical conditions (Strand et al., 1977; Gerritsen Van der Hoop et al., 1990) — important considerations reviewed by Gispen et al. (1994).

3. Regeneration studies

As we had determined that the melanocortins affected disturbed systems, it seemed logical to investigate a possible effect on peripheral nerve regeneration. The rate at which peripheral nerves regenerate varies with the species and the type of lesion induced. Following crush injury, the nerve sheaths are mostly still intact and there is, therefore, a better chance of reestablishment of the motor units than

Table 1
Structure of related ACTH peptides

		1	2	3	4	5	6	7	8	9	10	11	12	13
α-MSH	CH_3CO	-SER	-TYR	-SER	-MET	-GLU	-HIS	-PHE	-ARG	-TRP	-GLY	-LYS	-PRO	-VAL
ACTH 4–10					-MET	-GLU	-HIS	-PHE	-ARG	-TRP	-GLY			
ACTH 4–9					-MET	-GLU	-HIS	-PHE	-ARG	-TRP				
Org 2766				(O_2)	-MET	-GLU	-HIS	-PHE	D-LYS	-PHE	-OH			
BIM 22015				D-	-ALA	-GLN	-TYR	-PHE	-ARG	-TRP	-GLY	$-NH_2$		

after nerve transection. The earliest studies on the effects of ACTH peptides on nerve regeneration were done in our laboratory in 1980 (Strand and Kung, 1980). Administration of ACTH-(1–39) to adult, adrenalectomized rats accelerated the return of both sensory and motor function. These regeneration effects of the whole ACTH molecule were rapidly extended to structure–activity studies by Bijlsma et al. (1981), which showed that the neurotrophic effectiveness of this molecule could be restricted to the non-corticotropic fragments of the parent molecule: α-MSH (ACTH-(1–13), its smaller fragments ACTH-(1–10) and ACTH-(4–10), and the ACTH-(4–9) analog Org 2766. We have also used an ACTH-(4–10) analog, Biomeasure 22015 (BIM 22015) Table 1. With the availability of these smaller, non-corticotropic ACTH peptides it became possible to separate the peptide effects from the steroid-evoked actions of the whole molecule. These studies are discussed in much greater detail in several reviews (Strand et al., 1988, 1993a,b, 1996).

3.1. Morphological studies on the neuromuscular junction

The neuromuscular junction is an invaginated region of the muscle for entry and arborization of the nerve. At this site, the release of acetylcholine from the nerve terminal within the endplate elicits the mechanical response of the muscle to a stimulus. The formation of the mature neuromuscular junction depends on nerve and muscle interactions, with both tissues producing complementary trophic factors that guide the nerve to the muscle. Following nerve crush, the injured axons degenerate, to recover with new growth cones following the course of the old axon sheaths. Even though regenerating axons are unable to find the specific muscle fibers they originally innervated, they find old endplate sites with amazing accuracy, innervating existing endplates along the length of each fiber. However, reinnervation of crush-denervated muscle results in a radical change in the organization of the motor unit, since the muscle fibers of the individual motor units are concentrated in small regions instead of being widely dispersed (Fig. 2). The reinnervated endplate, which following nerve crush is depleted of nerve terminals, shows considerable increase in the preterminal branching and arborization after treatment with ACTH-(4–10), α-MSH or BIM 22015. Fig. 3 shows the marked improvement in the structure of the endplate after treatment with the ACTH-(4–10) analogue BIM 22015. (Strand and Kung, 1980; Strand et al., 1988, 1996).

3.2. Electrophysiological studies.

The pattern of reinnervation is considerably improved by the administration of the melanocortins, resulting in an increased number of smaller motor units (Fig. 2) and consequently finer motor control. ACTH-(1–39), ACTH-(1–13), ACTH-(4–10), Org 2766 and BIM 22015 all

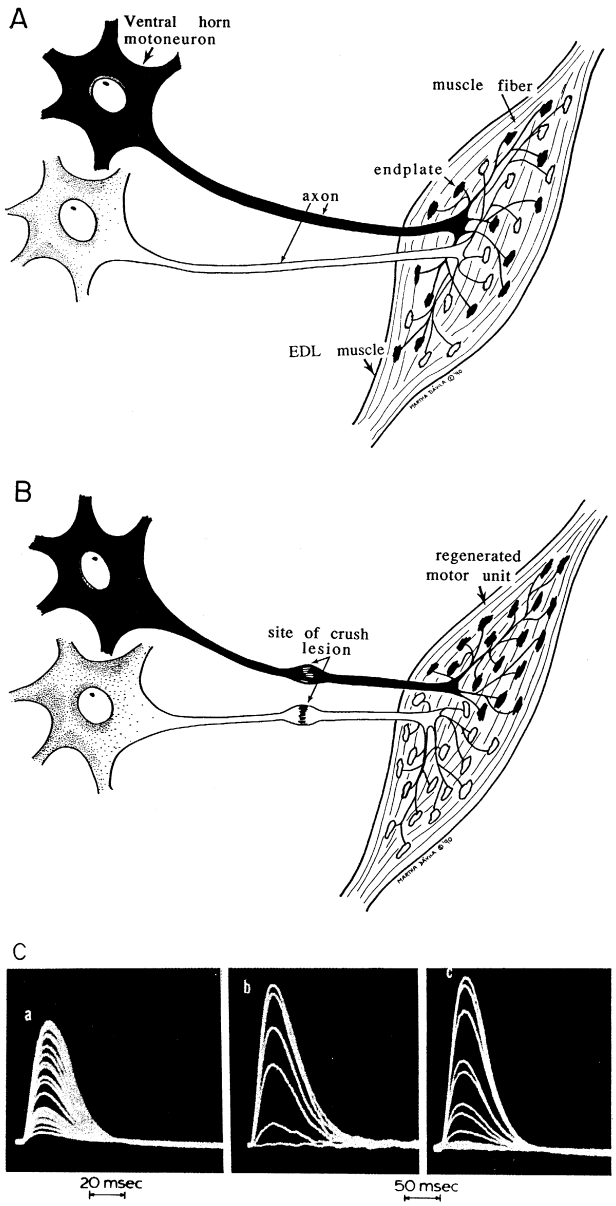

Fig. 2. (A) In normal extensor digitorum longus (EDL) muscle, widely distributed muscle fibers innervated by a single motor neuron form a motor unit. Two of ~18 motor units are shown. (B) After crush denervation regenerated motor units are large and the muscle fibers of each unit are closely clustered. Only ~6 motor units serve to innervate all muscle fibers of the EDL. Administration of ACTH-(4–10) from time of crush permits reinnervation in a pattern more like that of intact muscle, with motor unit size ~9–12, as shown in electrophysiological recordings in C. (C) recruitment of motor units in the EDL with finely graded increases in stimulus strength to peroneal nerve. a, Intact; b, denervated + saline; c, denervated + ACTH-(4–10) (10 μg/48 h) for 7 days. Motor unit recruitment in intact rats is characterized by small, discrete increments in twitches. Crush denervation results in large incremental steps in muscle twitches, indicative of fewer but larger motor units. Peptide administration increases the number of small motor units in reinnervated muscle. From Saint-Come and Strand (1985).

increase the speed of muscle contraction and decrease muscle fatigue during prolonged periods of stimulation in

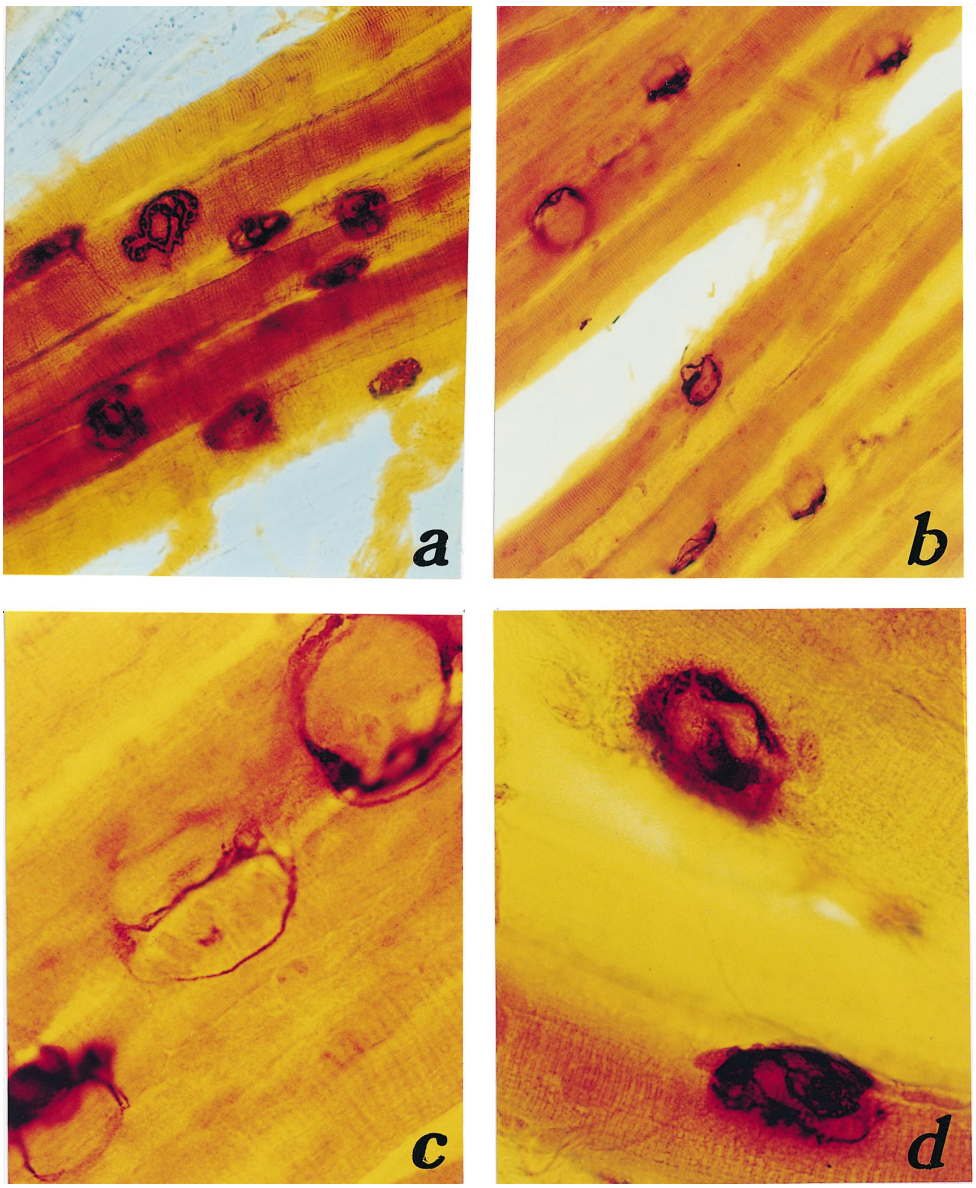

Fig. 3. Nerve terminal branching within endplates of rat extensor digitorum longus muscle 14 days after peroneal nerve crush. (a) Sham-denervated + saline, 200 ×; (b) nerve crush, saline-treated, 200 ×; (c) nerve crush, saline-treated, 600 ×; (d) nerve crush + BIM 22015 0.1 μg/kg, 600 ×. From Strand et al. (1994).

various animal models of altered metabolic states (Strand et al., 1976). These observations were confirmed and extended by nerve conduction measurements (de Koning and Gispen, 1987) and electromechanical studies (Saint-Come and Strand, 1985, 1988). It is important, however, to realize that these peptides do not increase physiological parameters beyond those of non-treated, intact animals.

3.3. Sensory and motor behavior

Adult, adrenalectomized rats subjected to sciatic nerve crush, recover both sensory and motor competence faster following daily injections of ACTH-(1–39) for 2 weeks (Strand and Kung, 1980). Motor function following sciatic nerve crush, as evaluated by the Sciatic Functional Index of De Medinaceli et al. (1982), is accelerated by treatment with either ACTH-(4–10) or α-MSH (10 μg/rat per 48 h) for 8 days (Fig. 4). However, peptide treatment continued for 21 days has a deleterious effect on both functional recovery and on electromechanical parameters of reinnervated muscle (Strand et al., 1993a). We have found interesting variations in the trophic properties of ACTH-(4–10) and its analogue BIM 22015. α-MSH and ACTH-(4–10) are more effective on the electrophysiological characteristics of denervated muscle, but BIM 22015 is considerably more potent on myotropic characteristics (Table 2; Strand and Kung, 1980; Strand et al., 1988, 1993a).

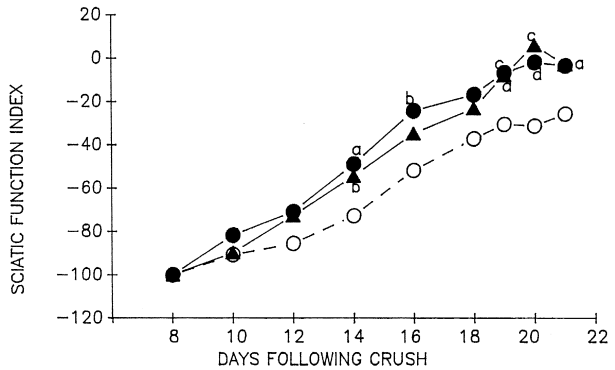

Fig. 4. A comparison of functional recovery among adult rats treated with saline ($n = 4$), open circles; α-MSH ($n = 6$), filled circles; ACTH-(4–10) ($n = 6$), filled triangles, 10 μg/kg per 48 h) for 8 days following sciatic nerve crush as measured by the Sciatic Functional Index. ANOVA: a = $P < 0.05$; b = $P < 0.025$; c = $P < 0.01$; d = $P < 005$. From Strand et al. (1993a).

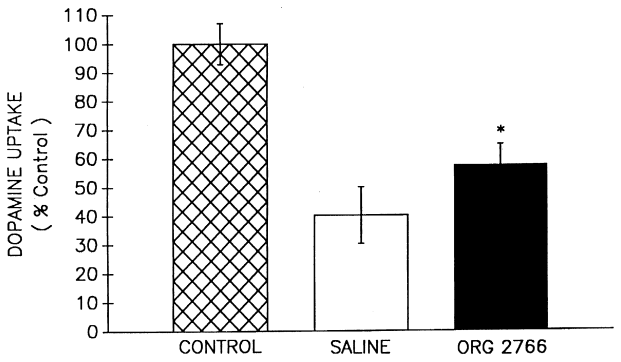

Fig. 5. Changes in specific high-affinity dopamine uptake (percent control) in the striatum 3 days after 6-hydroxydopamine lesions of the substantia nigra. The mean specific dopamine uptake was 17% higher in Org 2766-treated (10 μg/kg/24 h) animals as compared to saline controls, suggesting a higher fiber density in the Org 2766 group. Values for the intact contralateral striata (non-lesioned side) of each animal were normalized to 100% and are represented by the first "control" bar ($n = 7$). * $P < 0.05$. From Antonawich et al. (1994).

3.4. Neuroprotection in the spinal cord

Using immunocytochemistry we demonstrated that ACTH-(4–10) immunoreactivity is present endogenously in ventral horn motor neurons although in relatively low levels, probably due to circulating neuropeptides. Nissl staining confirms this motor neuron loss. Following unilateral peroneal nerve crush, saline-treated rats lose ACTH-(4–10) immunoreactivity ipsilateral to the lesion. This contrasts with the markedly increased ACTH-(4–10) immunoreactivity on both the ipsilateral and contralateral sides of the cord in lesioned rats administered ACTH-(4–10). We interpret this to indicate that exogenous ACTH-(4–10) may offer a neuroprotective action superimposed upon the low endogenous level of this peptide (Lee et al., 1994).

3.5. Neuroprotection in the brain

We have demonstrated highly specific immunoreactivity to ACTH-(4–10) in many regions of the rat brain during development and following central lesions (Lee et al., 1992; Antonawich et al., 1993b). In the intact adult brain, however, immunoreactivity is restricted to fibers of the arcuate and medial septal area. There is some evidence to indicate that the melanocortins affect regeneration in the central nervous system (Isaacson and Poplawsky, 1983; Nyakis et al., 1985) but these actions are more likely to involve compensatory events within the degenerated system. Collateral sprouting, increases in neurotransmitter

Table 2
Percent extensor digitorum longus muscle fiber diameter (as compared to sham crush as 100%) following the administration of peptides (10 μg/kg/48 h) 5 and 7 days after peroneal nerve crush

Time after crush	5 Days	7 Days
Crush and saline	57.0 ± 2.7	75.0 ± 2.0
Crush and BIM 22015	91.5 ± 3.7[a]	86.9 ± 3.5[b]
Crush and ACTH-(4–10)	70.9 ± 3.3	67.7 ± 2.2[b]

$N = 6$ rats per group; 240 muscle fibers per animal. Means ± S.E.
Only BIM 22015 values do not differ significantly from sham crush values. From Strand et al. (1993a).
[a] $P < 0.01$ vs. crush and saline.
[b] $P < 0.05$ vs. crush and saline.

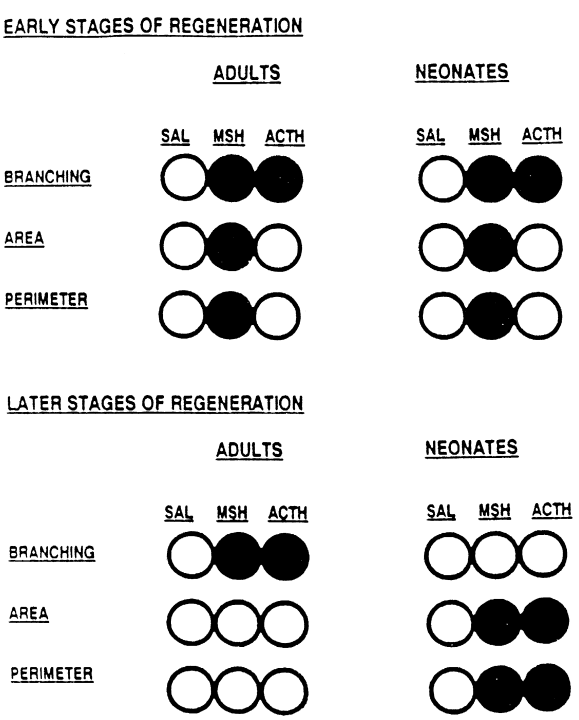

Fig. 6. A comparison of adult vs. neonate regeneration. During the early days of regeneration, new sprouts are susceptible to peptide influence, which enhances all aspects of the neuromuscular junction. During the later stages of regeneration, only the complexity of interior branching is affected in the adult, while in the neonate branching has reached its maximum but the area and the perimeter of the endplate are enlarged. From Zuccarelli and Strand (1991).

release from neighboring neurons or protection from further destruction may be actions promoted by the melanocortins. On the basis of studies showing that the melanocortins facilitate functional recovery in the nucleus accumbens (Wolterink et al., 1990), we investigated the action of the ACTH-(4–9) analogue (Org 2766) on the recovery of motor function in rats with neurotoxic lesions in the nigrostriatal system, which depletes the striatum of dopamine, a transmitter deeply involved in motor pathways. This treatment acutely enhances rapid behavioral, morphological and biochemical recovery 2 weeks after lesioning. As functional reinnervation is highly unlikely in this short time period, it appears that Org 2766 may be facilitating this recovery by either compensation or neuroprotection. To distinguish between compensatory mechanisms in this model and morphological evaluation of neuronal survival or regeneration following peptide treatment, immunocytochemical studies and evaluation of high-affinity dopamine uptake were undertaken. Both these techniques demonstrate a higher uptake of dopamine and an increase in fiber density in the peptide-treated animals (Fig. 5). We suggest that this indicates a protective action of Org 2766 as well as an acceleration of compensatory mechanisms involved in functional recovery in the central nervous system (Antonawich et al., 1993a).

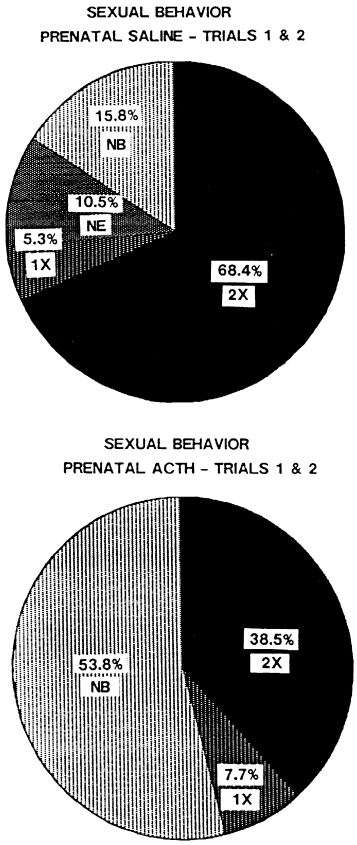

Fig. 8. Overall sexual behavior displayed by sexually experienced adult male rats treated prenatally with saline or ACTH-(1–24) (500 μg/kg 2×/day intraperitoneally to pregnant females, gestation days 14–21. 2× = percent males that completed at least two ejaculatory series; 1× = percent males that completed only one ejaculatory series per trial; NE = percent males that did not complete at least one ejaculatory series; NB = percent males that did not mount. Kolmogorov–Smirnov ($P < 0.05$). From Segarra et al. (1991).

4. Developmental studies

It is during the first 2 weeks after birth that the development of the neuromuscular system of the rat is finally completed. The non-corticotropic fragments of ACTH exert considerable influence over the maturation of neuromuscular system both in utero and during the formative 2-week postnatal period. These peptides act on the structure of the developing neuromuscular junction, accelerate the maturation of the electromechanical properties and enhance nerve–muscle integration to hasten adult motor behavior in neonatal animals.

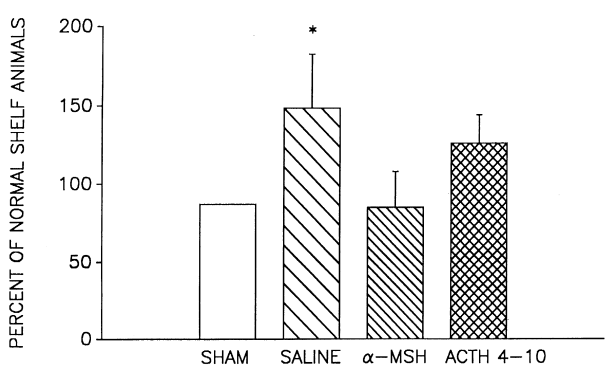

Fig. 7. A percent comparison of the time for 15-day-old neonates to lift both hindlimbs to a platform following sciatic nerve crush at postnatal day 2 and administration of α-MSH, ACTH-(4–10) (10 μg/kg/48 h) for 8 days, or saline vehicle. Sham animals received the same surgery as nerve-crush pups except for the crush. Normal shelf animals received no surgery (100%). Saline-treated, nerve-crush animals have significantly slower pull-up times as compared to all other groups. Nerve crush peptide-treated groups showed motor ability comparable to sham-crush and normal shelf groups. ANOVA $^{*}P < 0.001$. $n = 8$ for all groups. From Strand et al. (1993a).

4.1. Morphological changes

When viewed by scanning electron microscopy the endplates of 1- to 2-week-old rat pups are more complex, showing deeper junctional folds following the administration of ACTH-(4–10) (10 μg/kg/24 h) or of Org 2766 (0.01 μg/kg/24 h) (Frischer et al., 1985). Nerve terminal arborization is quantitatively increased when the nerve–muscle preparation is viewed with light microscopy following silver-acetylcholinesterase staining (Frischer and Strand, 1988). If the dosage of Org 2766 is raised to that of ACTH-(4–10), there is a considerable inhibitory effect on nerve fiber branching. As the synthetic analogue is 100× more potent than the equivalent dosage of ACTH-

(4–10) on certain parameters, this exemplifies the classic response to a peptide dosage that is too high for the particular system being investigated.

4.2. Electrophysiological responses

As a result of a more mature neuromuscular junction following chronic administration of ACTH-(4–10) (10 μg/kg/24 h) during gestation, the electromechanical responses are enhanced, with larger, faster contractions and greater resistance to fatigue after prolonged stimulation (Rose et al., 1988). The gestational effects of the peptide are complex since ACTH appears to give two time-dependent messages, first accelerating muscle development if administration is limited to early gestation (days 3–12); then modulating this development if administered during the later stages of gestation. The neuromuscular system is responsive only during a specific window of time and the window is open briefly, first for muscle, then for nerve (Rose and Strand, 1988).

4.3. Behavioral changes

Earlier development of the neuromuscular junction and accelerated electrophysiological maturity result in remarkable behavioral changes in neonates treated with ACTH peptides. Neuromuscular coordination is improved, and the normal development of hyperactivity occurs sooner (Rose et al., 1988; Acker et al., 1985).

4.4. Development of the serotonergic system

Central serotonergic (5-HT) neurons are intimately involved in certain motor behaviors, including both male and female sexual reflexes in the rat. ACTH peptides alter the developing 5-HT system in culture (Azmitia and de Kloet, 1987), and in vivo (Alves et al., 1993)

4.5. Regeneration during development

Ability to recovery from nerve injury varies considerably according to the age at which the injury is sustained (Fig. 6). Neonatal rats are most sensitive to nerve injury and our model was developed to determine the effects of ACTH peptides on 2-day-old rat pups subjected to sciatic nerve crush. Their recovery is then monitored in terms of morphological, electrophysiological, behavioral and metabolic characteristics.

Two weeks after nerve crush, both ACTH-(4–10) and α-MSH overcome the initial muscle atrophy resulting from denervation to the extent that muscle fiber diameters from 15-day-old lesioned neonates treated with melanocortins are comparable in size to normal, saline-treated pups of the same age. At this stage of development, the endplates of the peptide-treated lesioned animals are larger and contain more nerve terminal branching than the saline controls (Zuccarelli and Strand, 1990).

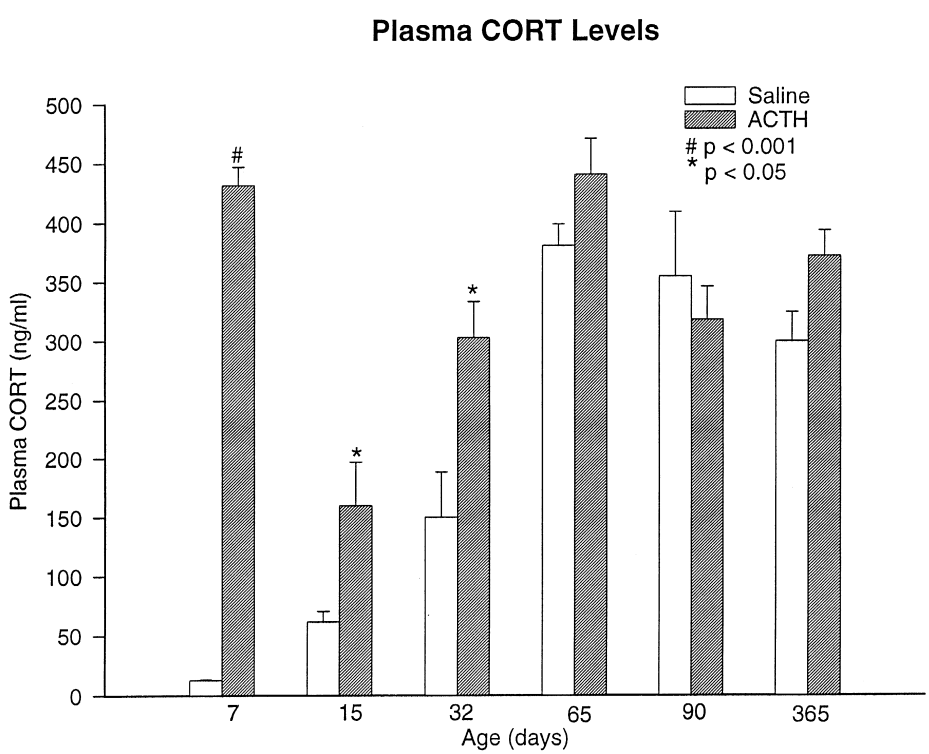

Fig. 9. Plasma corticosterone (CORT) levels following the last subcutaneous injection with either saline or ACTH (0.5 mg/kg/day) on day 7, and then "peak" basal levels at various ages following. Values are expressed as mg/ml ± S.E.M. (day 7, $n = 5$ [pooled]; days 15–365, $n = 5–7$). From Alves et al. (1997).

Both peptides accelerate reinnervation of the extensor digitorum longus muscle following sciatic nerve crush and this is accompanied by an improvement in the electrophysiological parameters, such as contraction amplitude, duration, and rate of rise. It is of considerable significance that α-MSH also increases the number of motor units, presumably restoring fine control in the traumatized, developing neuromuscular system (Zuccarelli and Strand, 1992).

A test for motor behavior in the neonate consists of having the pup pull itself up onto a platform by its forelimbs, then raise the hindlimbs to ascend the platform (Bregman and Kunkel-Bagden, 1988). Pups treated with ACTH-(4–10) or α-MSH do extremely well, requiring half as long to perform this task as saline-treated pups, and equaling the time required by uncrushed littermates (Fig. 7; Zuccarelli and Strand, 1991; Strand et al., 1993a).

4.6. Changes in hypothalamic–pituitary–adrenal axis and gonadal function

Perinatal manipulation of the hypothalamic–pituitary–adrenal axis by stress or by stress hormones such as ACTH

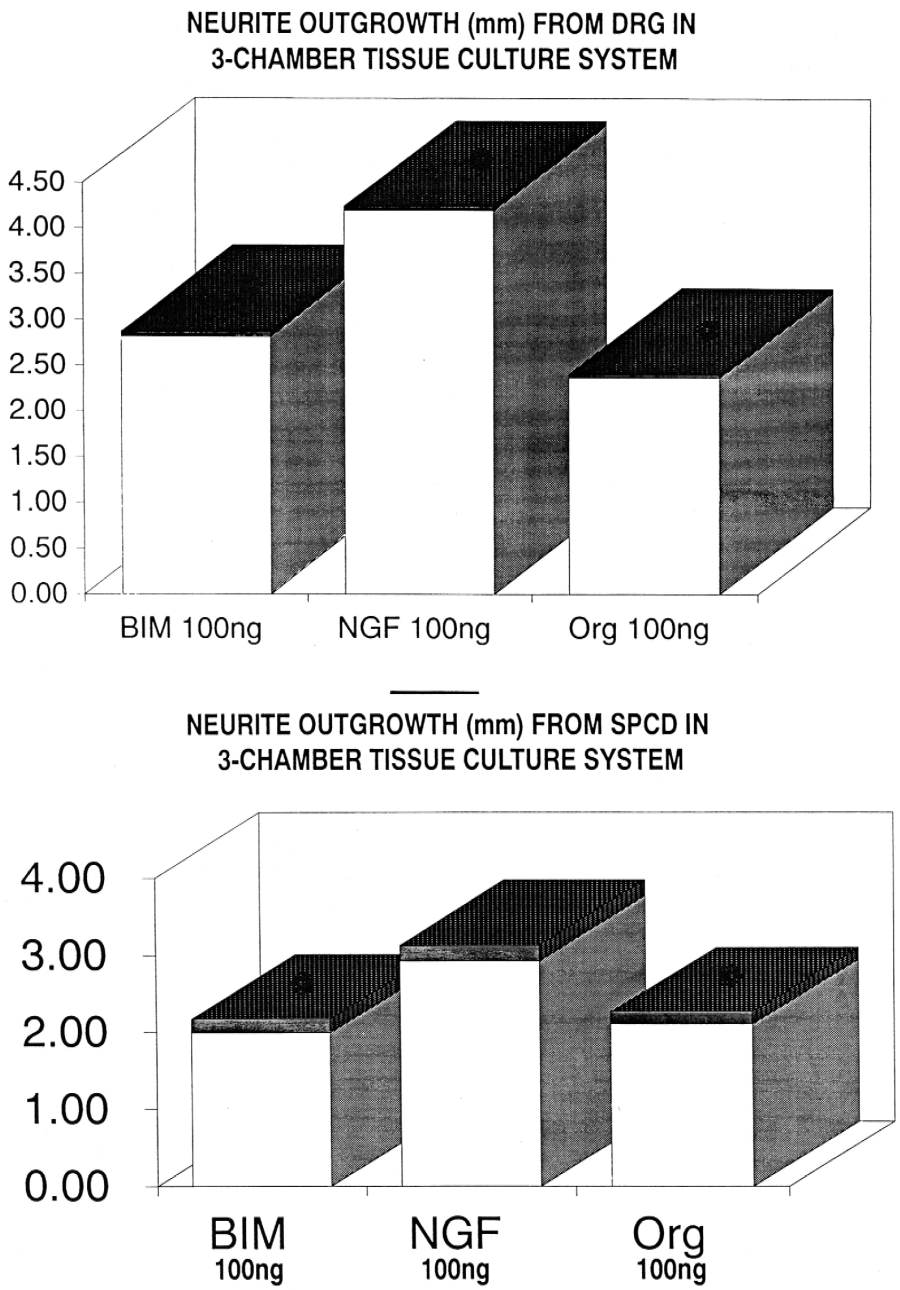

Fig. 10. The effects of ACTH analogues Org 2766 (ORG) vs. BIM 22015 (BIM) on neurite outgrowth from dorsal root ganglia (DRG) and spinal cord neurons (SPCD). DRG are more responsive to peptides than SPCD. BIM promotes more neurite extension than ORG ($P < 0.05$ for BIM vs. NGF vs. ORG. However, in the SPCD cultures, outgrowth with BIM does not differ significantly from that with ORG ($P < 0.02$ for BIM vs. NGF and ORG vs. NGF). From Strand et al. (1993a).

alters the development of central monoamine neurons (see Section 4.4). Our laboratory has been interested in the effects of ACTH peptides on the sexual differentiation of the brain and the role of the monoaminergic systems in this process. Late gestational treatment with ACTH decreases the expression of male sexual behavior in young adult male rats (Fig. 8), a change correlated with increased serotinergic input within the medial preoptic area, a primary regulatory site for male sexual behavior (Segarra et al., 1990, 1991). A long-term study in female rats ranging in age from 15 days to 1 year, of changes in the hypothalamic–pituitary–adrenal axis due to perinatal ACTH administration, indicates similar effects in the female rat brain, not only on the forebrain monoamine systems but also on adrenocortical activity (Alves et al., 1997). These animals respond to the mild stress of vaginal smears with a 30% increase in corticosterone levels (Fig. 9). The forebrain neuronal systems mature earlier and suggest an accelerated aging process. The hypothalamic–pituitary–gonadal axis is disrupted as well, resulting in a delayed onset of puberty (Alves et al., 1993) and decreased proestrus estradiol. Deficits in female sexual behavior are also manifested but they are not as marked as the sexual deficits seen in similarly treated males (Segarra, 1991). The clinical significance of these findings may be extensive since perinatal exposure to stress may have both acute and long-term deleterious effects on these neuroendocrine systems.

5. Melanocortins as growth factors

Using the three-chambered tissue culture system of Campenot (1977) we tested the neurotrophic properties of ACTH-(4–10), Org 2766 and BIM 22015 on dissociated, mixed spinal cord cells and compared the effects of these melanocortins with that of nerve growth factor (Fig. 10). While both ACTH analogues (Org 2766 and BIM 22015) promote neurite outgrowth in the absence of nerve growth factor ACTH-(4–10) surprisingly, does not. Nerve growth factor is more potent than either melanocortin in these cultures (Lee et al., 1991, 1992; Strand et al., 1993a). However, the ability of some melanocortins to enhance growth rate, even in the absence of nerve growth factor, correlates well with their remarkable effects in both regenerating and developing neural systems.

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