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Susceptibility of the Developing Neuroendocrine and Neuromuscular Systems of the Rat to Stress: Implications for Human Fetal Development

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Abstract

Perinatal exposure to the stress hormones adrenocorticotropin (ACTH) and cortisone profoundly affects the development of the neural circuitry responsible for reproductive function and behavior of both male and female rats. Hypothalamic monoamine innervation was increased in neonatal females administered ACTH 1-24 (0.5 mg/kg) daily for the first 7 days postnatal. Vaginal opening was delayed and peptide-treated animals displayed decreased female sexual behavior when tested as adults. It is suggested that the increased levels of hypothalamic serotonin, found in both males and females, following early exposure to stress hormones may have a long-term, inhibitory effect on adult reproductive behavior.

The neurotrophic actions of the non-corticotropic fragments of ACTH (ACTH 4-10, ACTH 4-9, ACTH 1-13 [α-MSH], and various analogs) may be separated from the stressful effects of ACTH 1-24. Maturation of the developing neuromuscular system is accelerated by early exposure to these short ACTH fragments. Morphological, biochemical, electrophysiological and behavioral studies all show that ACTH peptide fragments accelerate not only normal development but also regeneration in the traumatized, developing neuromuscular system. Dosage and timing of peptide administration are

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crucial as the plasticity of the neuromuscular system decreases with age. It is suggested that these ACTH peptides, devoid of harmful side effects, may be useful in certain human infantile neuromuscular disorders.

Zusammenfassung

Bei männlichen wie bei weiblichen Ratten führt die perinatale Einwirkung der Stress-Hormone Adrenocorticotropin (ACTH) und Cortison zu tiefgreifenden Auswirkungen auf die Entwicklung des neuralen Regelsystems, das für die Fortpflanzungsfunktionen und -verhaltensweisen verantwortlich ist. Die hypothalamische monoaminabhängige Innervation war bei weiblichen Neugeborenen, denen täglich ACTH 1-24 (0,5 mg/kg) während der ersten 7 Tage nach der Geburt zugeführt wurde, erhöht. Die Öffnung der Vagina war verzögert und die peptid-behandelten Tiere zeigten ein abgeschwächtes weibliches Sexualverhalten, wenn sie im Erwachsenenalter getestet wurden. Es wird angenommen, daß der erhöhte Wert von hypothalamischem Serotonin, der bei männlichen wie bei weiblichen Tieren nach frühzeitiger Behandlung mit Stress-Hormonen gefunden wird, eine hemmende Langzeitwirkung auf das Fortpflanzungsverhalten im Erwachsenenalter hat.

Die neurotropen Einflüsse von nicht-corticoiden Fragmenten von ACTH (ACTH 4-10, ACTH 4-9, ACTH 1-13 [α-MSH] und verschiedener Analoge) kann von den Stress-Effekten des ACTH 1-24 unterschieden werden. Die frühzeitige Behandlung mit diesen kurzen ACTH-Fragmenten beschleunigt die Reifung des sich entwickelnden neuromuskulären Systems. Morphologische, biochemische, elektrophysiologische und Verhaltens-Studien zeigen übereinstimmend, daß ACTH-Peptid-Fragmente nicht nur die normale Entwicklung beschleunigen, sondern auch die Regeneration des traumatisierten neuromuskulären Systems in der Entwicklungsphase. Dosierung und Zeitpunkt der Peptid-Zufuhr sind entscheidend, da die Plastizität des neuromuskulären Systems mit dem Alter abnimmt. Es wird angenommen, daß diese ACTH-Peptide, bei denen bisher keine schädlichen Nebenwirkungen bekannt sind, für die Behandlung einiger neuromuskulärer Erkrankungen im Kindesalter nützlich sein könnten.

Introduction

During development, both the neuroendocrine and the neuromuscular systems of the rat are susceptible to exposure to stress or to the stress-evoked hormone adrenocorticotropin (ACTH). In the rodent, the basic sexual differentiation of the brain, the gonads and subsequent adult mating behavior are organized by sex steroids in the latter part of gestation and early neonatal life. The inherent

neuronal pattern of the brain appears to be more "female-like", which develops into the acyclic male brain under the influence of the testosterone surge from the fetal/early postnatal testes; functional differentiation of the female rat brain is also believed to require some hormonal induction via low levels of estrogen (see review[2]). Exposure of the female brain to testosterone or elevated levels of estrogen during this critical period irreversibly transforms the cyclic female brain into the acyclic male type. The fundamental pattern of sexual differentiation of the developing brain can be seriously disrupted by exposure of the pregnant rat to stress, or by the administration of ACTH during specific critical times in gestation or early neonatal life. Male offspring of stressed mothers show decreased male sexual behavior as adults, accompanied by relatively high levels of female sexual behavior.[14,19] The deleterious effects of prenatal ACTH treatment in the male have been described in detail.[2,23] In this paper we review the changes in reproductive parameters in the female rat exposed perinatally to ACTH, reporting also on its effects on the development of hypothalamic neurotransmitter systems known to be sensitive to levels of ACTH peptides[1,3,8] and to titers of the adrenal steroids.[4]

Early experiments indicated that exposure to cold stress increased the amplitude of peripheral nerve action potentials and muscle contractions in adult rats, and that this was a direct effect of ACTH, not mediated via the adrenal cortex.[24] The purely neurotrophic effects of ACTH can be separated cleanly from its corticotropic actions through the availability of non- corticotropic fragments, neurotrophic factors that are limited to the amino acid moieties contained within the sequence ACTH 1-13 (α-melanocyte stimulating hormone [MSH]). These fragments are collectively called melanocortins and the most extensively investigated sequences include ACTH 4-10, its analog BIM 2766, the ACTH 4-9 analog (Org 2766) and α-MSH, all of which are devoid of adrenocorticotropic activity. The corticotropic activity of the molecule is contained within the 11-24 amino acid sequence of ACTH 1-24; the remaining 25-39 sequence of the endogenous ACTH 1-39 molecule appears to enhance its binding capacity.

The developing neuromuscular system is extraordinarily sensitive to the stress hormone ACTH and to its non-corticotropic fragments. In the rat, neuromuscular maturation continues through the first 2 weeks of neonatal life, involving replacement of polyneuronal innervation by mononeuronal innervation, increased synthesis of acetylcholine, acetylcholinesterase and their receptors, and the biochemical and electrophysiological changes characteristic of mature muscle fibers. These processes are accelerated by exposure to ACTH or to peptide fragments of this polypeptide hormone.[16] The neurotrophic action of the melanocortins has been extensively demonstrated by regeneration studies in adult rats: melanocortins accelerate both sensory and motor nerve regeneration, improve the pattern of motor unit formation in reinnervated skeletal muscle, normalize the morphology of the endplate and permit the more rapid return of both motor and sensory responses (see reviews[5,25–28,30]). In this paper, we review the evidence that both the normally developing, and the injured, developing neuromuscular system of neonates are positively affected by the melanocortins.

A. Stress and the Sexual Development of the Female Brain

Materials and Methods

Female Sprague Dawley rat pups were injected s.c. from day 1 to day 7 with either ACTH 1-24 (0.5 mg/kg) donated by Organon Inc., or 0.9 % saline, once daily. Hypothalamic synaptosomal high-affinity specific uptake was measured to assess hypothalamic monoamine neurotransmitter fiber density. Specific serotonin (5-HT) and dopamine (DA) uptake were measured at day 7 (neonatal), day 25 (juvenile) and 80–90 days (adult, during diestrus). [1]

Reproductive parameters measured included day of vaginal opening as an indication of reproductive maturation, and the evaluation of female sexual behavior. Between 60 and 70 days of age the animals were tested as intact virgins and placed in contact with sexually experienced adult males. The lordosis quotient (LQ), a quantitative measure of the number of lordotic responses of the female, and the lordosis quality score (LQS) a measure of the intensity of the lordotic response, were determined. [1]

Plasma corticosterone levels were determined on postnatal day 4, 45 min after the injection of ACTH 1-24 (0.5 mg/kg or 0.1 mg/kg), or saline to observe adrenal response to these treatments. Plasma estrogen and progesterone levels were determined in adults during late proestrus. All steroids were measured using Coat-a-Count radioimmunoassay kits from Diagnostic Products Corporation. Data were analysed by the Student's t-test. Significance was determined at $p < 0.05$.

Results

Neurotransmitters. As shown in Fig. 1, the ACTH-treated females had significantly higher specific 5-HT uptake in the hypothalamus on days 7 and at adulthood, suggesting increased fiber outgrowth at these stages in development. This increase was not seen in juveniles at day 25 postnatal. Similarly, specific DA uptake was higher in the peptide treated rats at day 7 ($p < 0.01$) and at adulthood, although the latter value was not significant. [1]

Reproductive Parameters. Vaginal opening was delayed in the ACTH-treated females ($n = 21$) from 34.7 ± 0.8 in the saline controls ($n = 20$) to 38.0 ± 0.3 days postnatal ($p < 0.01$). The peptide-treated animals displayed decreased female sexual behavior, having lower LQs and LQSs compared to saline treated animals when tested as young virgins (Figs. 2 and 3).

Plasma Steroid Levels. The ACTH-treated animals showed a dose-dependent increase in plasma corticosterone levels at postnatal day 4. Saline-treated rats had unrecordable levels of the stress hormone, suggesting that handling and injection stress were insufficient to elevate levels of endogenous ACTH and hence corticosterone in the control animals (Table 1). There were no statistical differences in plasma levels of the ovarian steroids following ACTH treatment.

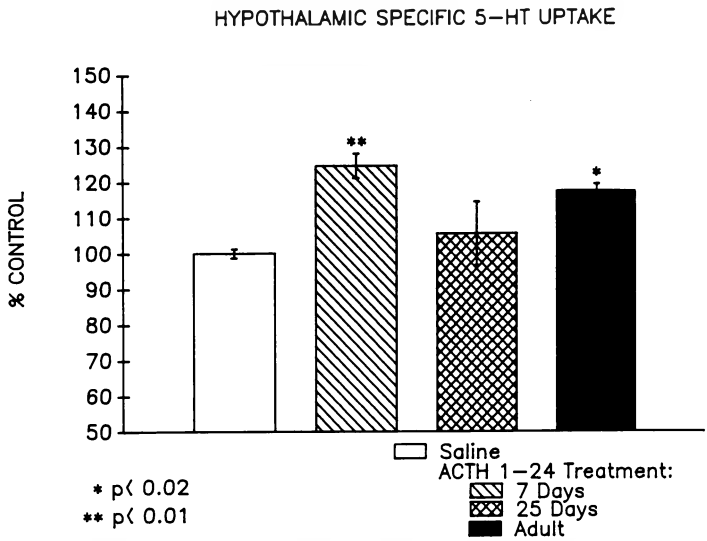

Fig. 1. Changes in high-affinity specific serotonin (5-HT) uptake (percent of control) in the hypothalamus of 7 day, 25 day and adult (80–90 day) rats following daily ACTH 1-24 injections (s.c., 0.5 mg/kg) during the first week of postnatal life. Values for saline treated animals (control) at each age were normalized to 100 percent and are represented by one bar (error bar is mean of the errors over the three time points, n = 4 per subgroup). (From Alves et al., 1993)

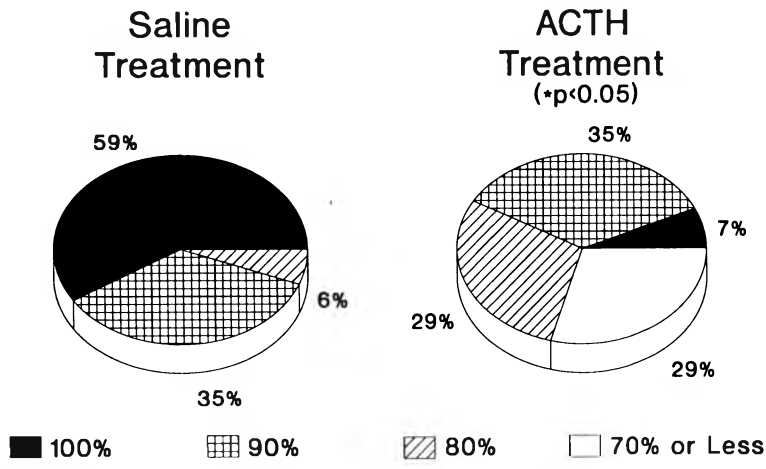

Fig. 2. Percent distribution of lordosis quotients among saline and ACTH 1-24 (0.5 mg/kg/day) treated animals during the first week postnatal (n = 17 per group) tested as intact virgins at 60–70 days of age. The percent of ACTH treated animals achieving an LQ of 90–100 is only 42 % where as about 94 % of the control animals achieved scores within this range ($p < 0.05$). (From Alves et al., 1993)

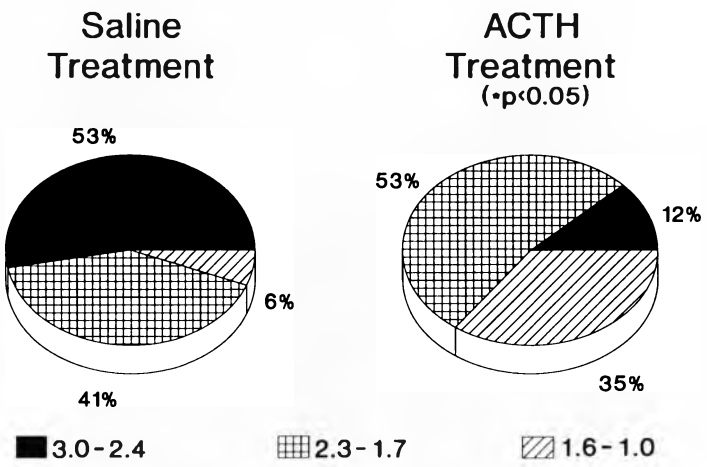

Fig. 3. Percent distribution of mean lordotic quality scores of saline and ACTH 1-24 (0.5 mg/kg/day) treated animals during the first week postnatal (n = 17 per group) tested as virgins at 60–70 days of age. The percent of saline-treated females achieving LQS's in the highest range (2.4–3.0) is 53 %, a level achieved by only 12 % of the treated rats ($p < 0.05$). (From Alves et al., 1993)

Table 1. Plasma Corticosterone levels on postnatal day 4 following daily sc injections with saline or ACTH 1-24.

Treatment	Plasma Corticosterone (ng/ml)
Saline	n.m.
ACTH (0.1 mg/kg/day)	331.8 ± 12.8
ACTH (0.5 mg/kg/day)	586.9 ± 31.4

Plasma was collected 45 minutes following sc injection with either saline or one of the two dosages of ACTH on postnatal day 4. Each value is the mean ± SE of pooled samples from 5 animals per group (n.m. = not measurable).

Discussion

Perinatal stress can disrupt the sexual differentiation of the rodent brain, most probably throughan alteration in the neuronal circuitry responsible for reproductive function and behavior, both male and female. The serotonergic system appears to play an integral part in this process and the extent of these changes is dependent upon the time of exposure and the sex of the animal. Hypothalamic monoamine innervation was increased in neonates by early postnatal exposure to ACTH and corticosterone. This amplified growth of the serotonergic system was diminished in the juvenile stage when considerable neuronal reorganization and synaptic formation was occurring, but the long-lasting effects of ACTH treatment became visible in early adulthood when both 5-HT and DA innervations were enlarged.[1]

Reproductive parameters declined in ACTH-treated females as seen in the delayed vaginal opening and decreased LQ and LQS scores. While exposure to elevated levels of ACTH and corticosterone during critical periods in development did not eradicate the expression of receptivity in the adult female, rejection of the male, accompanied by distress sounds, was observed among many of the stress hormone-treated females, a behavior rarely seen in the control animals.

Correlation of these deficits with the increased levels of the monoamines 5-HT and DA resulting from ACTH administration, is tempting. Both of these neurotransmitters have been reported to have a tonic inhibitory effect on female reproductive behavior, probably activating receptors in the medial basal hypothalamus and the ventromedial nucleus.[9,11,29] As these altered transmitter levels and the changes in reproductive parameters were observed in adult animals, previously treated as neonates with ACTH, we must assume that this stress hormone may have long term effects on the subsequent sexual behavior of the adult.

B. Stress and the Developing Neuromuscular System

Materials and Methods

For the morphological and behavioral studies on rat neonates, Sprague-Dawley litters were culled at birth to eight and the pups randomized for the following treatment groups: i) 0.9 % saline; ii) ACTH 4-10, 10 μg/kg; iii) and iv) Org 2766, the trisubstituted analog of ACTH 4-9, 10 μg or 0.01 μg/kg. All injections were administered s.c. daily from day 1. The animals were sacrificed at 7, 14 or 21 days of age.

For the electrophysiological studies, which were concerned with the specific period during gestation that was critical for melanocortin influence on the developing neuromuscular system, the dams were divided into 3 groups which received ACTH 4-10 (10 μg/kg/ 2 $\times$ day i.p.) as follows: i) from gestational days G3-21 ii) from G3-12 followed by saline iii) from G13-21, preceded by saline from G3-12. A fourth group received daily injections of saline from G3-21. Testing for electrophysiological parameters began on the resulting pups during days 14–15 postnatal.[18] In a different study, neonatal pups were administered ACTH 4-10 (10 μg/kg/24 h s.c.) on day 1 postnatal and every day thereafter for 7, 11 or 15 days at which time electrophysiological parameters were investigated.[20,21]

Parameters Measured

Morphology and Biochemistry. Using light microscopy and digitizing morphometry, the area, perimeter and internal branching of the neuromuscular junction (nmj) of the peroneal nerve and extensor digitorum longus (EDL) muscle, as visualized by silver-acetylcholinesterase stain, was quantified. The diameters of the muscle fibers were measured. Each group consisted of 40 nmjs and measurements were made with an image analysis system and the data and outline graphics were fed into a computer. The Student t-test was used for statistical

evaluation. In the series treated with the 2 dosages of Org 2766, the endplates were examined by scanning electron microscopy. [12]

The oxidative capacity of the reinnervated muscle was determined by the spectrophotometric analysis of insoluble dyes formed by the action of mitochondrial enzymes. [17]

Electrophysiology. Nerve-muscle relationships were studied by both direct and indirect electrical stimulation of the extensor digitorum longus (EDL) muscle via either the sciatic or peroneal nerve, with the pups under sodium pentobarbital (40 mg/kg/ i.p.) anesthesia. Motor unit recruitment and the characteristics of the twitch and tetanus were measured, including resistance to muscle fatigue, muscle strength and speed of muscle contraction and relaxation. [16,18]

Behavioral Correlates. Spontaneous motor activity was recorded with an animal activity monitor. Stress-induced motor activity was examined in the neonates by placing the pup on a cold metal plate and determining the latency time for escape from the plate. [20] Grasping time evaluation involved the time the animal was able to grasp a horizontal bar while suspended This is a quantitative evaluation of forelimb strength and the grasping reflex, and thus may be considered an indication of neuromuscular maturation. [21]

Results

ACTH 4-10 (10 μg/kg) stimulated nerve terminal branching in 7 and 14 day-old pups. The lower dosage of Org 2766 (0.01 μg/kg) was more effective than 10 μg/kg ACTH, increasing both branching and endplate perimeter (Table 2). Interestingly, the higher dosage of Org 2766, which was the same as that of ACTH 4-10 i.e. 10 μg/kg), markedly inhibited sprouting. This U-shaped dose-response is characteristic of the melanocortins, higher dosages usually reversing or preventing the stimulatory effect of the lower dosage.

Table 2. Endplate measurements from EDL muscles of rats treated with ACTH/MSH 4-10 (10 μg/kg) or Org 2766 (0.01 μg/kg/day) daily from day of birth.

	Muscle fiber diameter [μm]	Endplate area [μm^2]	Endplate perimeter [μm]	Nerve terminal branching [μm]
7 Days of age				
Saline	32.1 (0.7)	1010.8 (42.1)	126.1 (2.7)	108.3 (3.9)
ACTH	29.4 (0.5)***	907.2 (27.7)*	122.3 (2.2)	94.6 (3.8)*
Org 2766	32.9 (0.9)	1084.0 (40.6)	137.7 (2.5)**	122.2 (4.1)*
14 Days of age				
Saline	38.2 (0.9)	1556.8 (82.9)	160.5 (4.0)	146.4 (6.7)
ACTH	38.2 (0.9)	1631.9 (63.9)	159.0 (2.9)	172.7 (8.0)**
Org 2766	38.5 (0.9)	1792.9 (105)	166.9 (4.8)	192.7 (7.7)***

N = 40, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Means (S.E.M.).
Data from Rose et al. (1988) and Frischer and Strand (1988).

Normal maturation of fast muscle fibers, such as the EDL, involves a switch from oxidative to glycolytic metabolism, a switch that was accelerated in the peptide-treated animals.[17]

Electrophysiological studies of pups exposed to ACTH 4-10 during various periods of gestation, and tested as neonates, showed that ACTH 4-10 had a positive effect on developing muscle during the first part of gestation GD 3-12. Subsequently, as the EDL became innervated, muscle permanently lost its responsiveness to the peptide.[18] Neonates exposed to ACTH 4-10 during the first 2 weeks of life, however, showed markedly increased muscle strength and speed and increased resistance to fatigue, all changes indicating accelerated maturation. These effects were shown to be due to a direct effect of the peptide on the nerve and neuromuscular junction. By the third week, however, there were no significant differences between the groups.[21]

The behavioral studies indicated that whereas there was no difference in the spontaneous motor activity of peptide-treated and saline-treated pups, after cold-stress the Org 2766 treated rats were significantly more active than the controls. In this test of motor function, Org 2766 was considerably more potent than ACTH 4-10.[20] Grasping time was markedly lengthened by the administration of the peptides in 11–13 day old rats, an observation that correlates well with the increased muscle strength demonstrated in the electrophysiological studies.[21]

C. Stress and Regeneration of the Developing Neuromuscular System

Materials and Methods

Sprague-Dawley litters were culled at birth to eight and the pups randomized for distribution into the following treatment groups: normal animals (no treatment at all); sham-operated controls (same surgical procedure as peptide-treated groups but the sciatic nerve was not crushed); saline-crushed controls (lesioned and administered saline); α-MSH crushed pups (lesioned and administered 10 μg/kg s.c.) every 48 h for 6–8 days; ACTH 4-10 crushed pups (lesioned and administered 10 μg/kg ACTH 4-10 s.c. every 48 h for 6–8 days). The sciatic nerve crush was performed on 2-day-old pups, under hypothermic anesthesia.

Parameters Measured

Morphology, Biochemistry and Electrophysiology. Essentially the same as for the developmental studies in B.

Behavioral Correlates. A test for motor behavior in the neonate consisted of having the pup pull itself up on to a platform by its forelimbs, then raising the hindlimbs to ascend the platform.[6] Tests were carried out on days 15, 18, 21, 27, 30 and 35.

Results

ACTH 4-10 and α-MSH have potent positive effects on the morphology and biochemistry of the reinnervated EDL of the neonate. Interior endplate branching was increased by ACTH 4-10; α-MSH not only improved this parameter but

also increased endplate area and perimeter. By 2 weeks of age, both peptides increased all endplate measurements except interior branching which apparently had reached its maximum by this time (Tables 3 and 4). Muscle fibers atrophied following neonatal denervation: treatment with ACTH 4-10 but not with α-MSH exacerbated the atrophy at 7 days. However, by 15 days all peptide-treated muscles of the lesioned animals were comparable in size to normal, saline-treated pups of the same age.

Table 3. Endplate morphology and muscle fiber diameter 15 days after sciatic nerve crush (48 h postnatal) and peptide treatment (10 μg/kg/48 h/8 d).

	Saline	α-MSH	ACTH 4-10
Branching [μm]	128.6 ± 7.3	128.9 ± 3.6	122.6 ± 4.2
Area [μm^2]	1251.0 ± 86.1	1720.5 ± 31.2***	1575.0 ± 35.7***
Perimeter [μm]	153.0 ± 7.1	166.2 ± 1.9**	162.2 ± 2.2**
Diameter [μm]	32.5 ± .7	40.4 ± .5***	38.5 ± .6***
# Animals	4	5	7
# NMJ and Fibers	29	138	105

All visible endplates and their associated muscle fibers were measured.
ANOVA: saline vs peptide * $p < 0.1$, ** $p < 0.01$, *** $p < 0.0005$.

Table 4. Endplate morphology and muscle fiber diameter 7 days after sciatic nerve crush (48 h postnatal) and peptide treatment (10 μg/kg/48 h/6 d).

	Saline	α-MSH	ACTH 4-10
Branching [μm]	75.2 ± 4.0	123.1 ± 7.2***	125.3 ± 4.2***
Area [μm^2]	1389.8 ± 54.3	1628.3 ± 81.3*	1352.8 ± 49.5
Perimeter [μm]	157.0 ± 4.0	175.1 ± 5.9#	159.3 ± 3.3
Diameter [μm]	32.5 ± .7	30.1 ± .9	28.8 ± .7**
# Animals	5	3	4
# NMJ and Fibers	53	25	39

All visible endplates and their associated muscle fibers were measured.
ANOVA: saline vs peptide * $p < 0.1$, ** $p < 0.002$, *** $p < 0.0001$, # $p < 0.02$.

The electrophysiological evidence supported peptide-accelerated reinnervation of the EDL. Treatment with α-MSH resulted in 50 % of the lesioned pups responding to electrical stimulation 13 days postlesion, as compared to a response of the ACTH 4-10 pups at 19 days, and saline- treated pups at 33 days postlesion. The melanocortins also permitted the reinnervated muscles, tested 21 days after nerve injury, to reflect normal contractile parameters of the twitch and tetanus (force amplitude, contraction rate, half-relaxation time, post-tetanic twitch amplitude and rate of rise). There was a significant increase in the number of motor units formed under α-MSH treatment (unpublished).

In the behavioral test for motor function, the lesioned pups treated with either ACTH 4-10 or α-MSH performed as effectively as the uncrushed controls, whereas the saline-treated, nerve-injured pups required twice as long to perform the tasks (Fig. 4). From observations of the video recordings, it appeared that the peptide-treated pups climbed the platform more rapidly, as they not only moved more quickly but their placing reactions were more accurate.

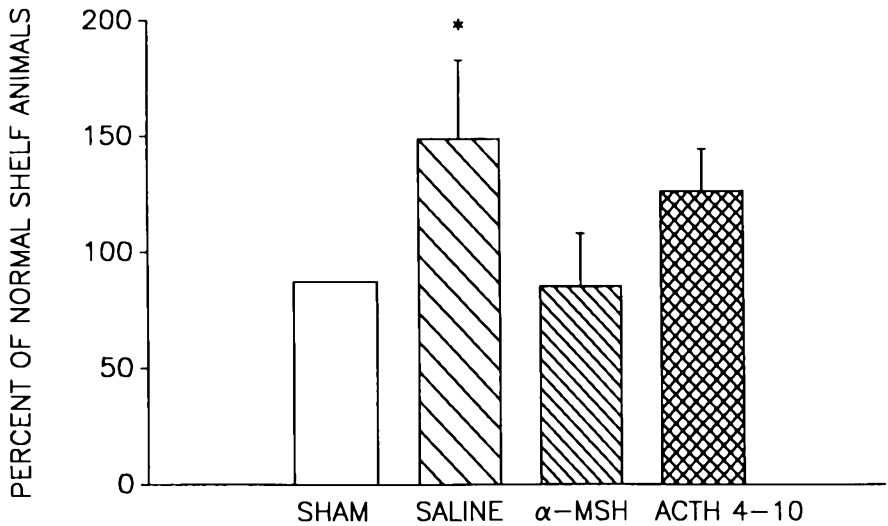

Fig. 4. A percent comparison of the time for 15 day old neonates to lift both hindlimbs to a platform following sciatic nerve crush at postnatal day 2 and administration of α-MSH, ACTH 4-10 (10 μg/kg/48h/8d s.c.) or saline vehicle. Sham animals received the same surgery as nerve- crush pups except for the actual nerve crush. Normal shelf animals received no surgery at all (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: * $p < .001$. $n = 8$ for all groups.

Discussion

Both the morphological and biochemical studies demonstrate that the melanocortins accelerate neuromuscular development. This conclusion is supported by the electrophysiological studies, which show that the effectiveness of the melanocortins on the developing neuromuscular system is dependent upon the time at which the developing nervous system is exposed to their action. When these fragments are administered to neonatal rats, their positive effects can be elicited only during the first 2 weeks of life, a time during which maturation of the neuromuscular system was occurring. More detailed analysis shows that there is a similar critical prenatal period of susceptibility, with embryonic muscle responding only prior to innervation. All subsequent melanocortin effects are neurotrophic.

It is interesting to note that the injured, developing neuromuscular system is similarly susceptible to the positive effects of melanocortins. Even in the face of trauma, melanocortins accelerate muscle fiber maturation, and restore normal neuromuscular integrative functions. Not only is the ontogenetic time of exposure to melanocortins critical but the dosage is decisive. Too low a dosage has little if any effect, too high may be inhibitory or even reverse the positive effects of the optimal dosage. [10] The mechanism by which the optimal level of endogenous melanocortins is maintained during specific periods of development is unknown.

Implications for Human Fetal Development

These investigations indicate that perinatal ACTH administration induces permanent changes in the hypothalamic-pituitary axis of the rat. In males, prenatal, but not postnatal, treatment results in long-lasting decrease in male copulatory activity, a deficit that also can be evoked by the prenatal administration of nicotine, [22] a drug that induces the secretion of endogenous ACTH. In females, postnatal ACTH administration induces deficits in female sexual behavior but the deficiency is less severe than in males, prenatally adminstered this peptide. In the rat, masculinization of the brain occurs mainly prenatally, whereas feminization occurs postnatally. [15] To extrapolate to humans in terms of gestation time is difficult but what is clear is that exposure of the pregnant or lactating woman to stress and/or cigarette smoking evokes the risk of permanently affecting the neuroendocrine axis of the fetus. Our experiments, using ACTH 1-24 in this part of the study, do not permit the separate evaluation of the role of the adrenal corticoids in this neuroendocrine response.

However, the studies on the neuromuscular system clearly indicate that it is the independent action of the ACTH peptide fragments (ACTH 4-10, Org 2766 and α-MSH) that accelerate neuromuscular development, enhance neuromuscular integration and facilitate nerve regeneration even during the fragile period of neuromuscular maturation. As clinical administration of these melanocortins to humans has been shown to be devoid of unwanted side- effects, [13] it is proposed that these hormones might be beneficial in certain neuromuscular diseases of infancy and childhood, such as in Charcot-Marie-Tooth syndrome, infantile myotonia and infantile spinal muscular atrophy. Suggestions from pediatricians would be welcome.

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Comment

Comments on F.L. Strand, L.A. Zuccarelli, and S.E. Alves: "Susceptibility of the Developing Neuroendocrine and Neuromuscular Systems of the Rat to Stress: Implications for Human Fetal Development"

J. C. Martinez

This excellent study by Fleur L. Strand and her co-workers reminds neonatologists once again of the powerful impact stress has on fetal development.

A premature infant is, in essence, a "displaced fetus" (Als, Brazelton) with a brain and nervous system that are highly sensitive and vulnerable to sensory input. The child is abruptly transferred from a warm, maternal environment to an intensely bright, noisy, newborn care unit. He is placed on a flat surface with wires and tubes attached to his fragile body and usually requires support for respiration as well as thermoregulation and nutrition. This transition to extrauterine life has a great impact upon premature infants, not only due to the abnormal physical environment of the NICU (Neonatal Intensive Care Unit), but also because of the painful procedures and excessive handling it involves.

The NICU has become a vigorous, therapeutic nursery in which preterm infants can be maintained successfully for several months improving their respiratory and metabolic condition. Unfortunately with the increase of therapeutic knowledge and such vigorous treatment, we have failed to develop at the same time knowledge of the psychological needs of these infants. The majority of VLBW (Very Low Birth Weight) infants may benefit from care procedures that diminish stress due to manipulation and that allow for longer periods of rest without disturbance.

It has been suggested that we should not eliminate stress altogether as it may be an important impetus for growth, but that it is equally important to decrease infant stress while simultaneously enhancing and facilitating his self-regulating abilities. It is possible to use the infant's behavior as well as the traditional physiological measures (H. Als) to monitor whether medical, nursing or social interventions are exceeding the infant's threshold for stress.

The excellent studies of neurobehavioral infant-child studies from Enders Pediatric Research Laboratories in Boston directed by Professor Heidelise Als, demonstrate a dramatic improvement of the outcome of the preterm baby's condition when behavioral observation was used in restructuring the environment and modifying care-giving techniques to avoid stress. The challenge is to optimize the outcome of the infant's development while providing the appropriate care.

The nursing staff could play a vital role in reducing stress within the neonatal environment by teaching parents to recognize and understand stress and its impact on the infant. Babies should not be considered passive participants and we should make every effort not to disturb their biological rhythms. The NICU at present is not the environment we would prefer if real goals in human care are to be achieved. We must go beyound infant survival and reach for optimal growth and development.

Basic research as Dr. Strand's study is a red light for neonatologists dealing with long-term effects of stress and should encourage us to promote more sensitive, appropriate and individualized care for our patients even with the resources available in our outdated nurseries.

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