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An ACTH/MSH(4-9) Analog Counteracts the Behavioral Effects of a Mineralocorticoid Receptor Antagonist

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OITZL, M. S., M. JOSEPHY AND B. M. SPRUIJT. *An ACTH/MSH(4-9) analog counteracts the behavioral effects of a mineralocorticoid receptor antagonist.* PHARMAC. BIOCHEM. BEHAV. 44(2) 447-450, 1993.—The ACTH/MSH(4-9) analog Org2766 has been demonstrated to counteract age-related behavioral and morphological parameters especially those related to hippocampal functioning. Hippocampal mineralocorticoid receptors (MRs) are known to decline in the senescent rat. This decrease can be also counteracted by a chronic treatment with an ACTH(4-9) analog. The apparent effect of the peptide on hippocampal functioning prompted us to study a possible interaction between ACTH and MRs at a behavioral level. A chronic treatment with the ACTH(4-9) analog prevented the behavioral alteration induced by a specific MR antagonist (RU28318; 100 ng/μl, ICV) in the Morris water maze and a step-through avoidance task. A possible role for neuronal excitation, involving MR activation, in peptide-facilitated behavioral recovery as seen in lesion studies and aging is discussed.

ACTH/MSH(4-9) analog Mineralocorticosteroid receptor antagonist Spatial learning
Inhibitory avoidance Rat

CHANGES in the function of the hippocampus underlie a substantial fraction of age-related impairment of behavior (14). The efficacy of the synthetic ACTH(4-9) analog (Org2766) in delaying age-related behavioral changes, especially the age-related decline in spatial learning and social interaction, has been demonstrated (22). A comparable chronic treatment of old rats counteracted a morphological index of aging of hippocampal neurons (13) and the age-related decline of mineralocorticoid receptors (MRs) (19). Also in fimbria fornix lesioned young rats chronic ACTH(4-9) treatment facilitated the recovery of spatial learning in the Morris water maze (17,25). In these studies it was suggested that neuronal reinnervation of the denervated structures is unlikely the underlying mechanism of functional recovery.

Corticosteroids play an important role in adaptation. They differentially occupy central MRs and glucocorticoid receptors (GRs), which mediate coordinated and differential effects of corticosteroids on the regulation of cellular homeostasis and behavioral adaptation (6). Alterations of MR and GR capacity in limbic structures occur in relation to stress and aging (6,15,21). Chronic infusion of the ACTH(4-9) analog (org2766) reversed the decrease of hippocampal MRs, but not GRs, in aged rats and increased the number of hippocampal MRs in young rats (19,20). Corticosteroids are involved in spatial learning as well. For example, the blockade of MR-mediated effects of corticosteroids by a selective MR antagonist was accompanied by altered search strategies of rats in the Morris water maze (16).

Thus, morphological and behavioral data point to an interaction between the ACTH(4-9) analog and MR activity in senescence and, MR activity is involved in those tasks studied in peptide facilitated recovery. The question is raised whether peptide treatment stimulates MR-activity. An enhanced stimulatory activity may facilitate functional recovery in lesioned and aged animals. One experimental approach to further examine this interaction is blocking the activity of central MRs by injecting a selective MR antagonist in young rats, which excludes interference of other age-related phenomena. We tested whether a chronic ACTH(4-9) pretreatment would protect against the effect of MR blockade on a spatial learning and inhibitory avoidance task.

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METHOD

Animals

Male Wistar rats (n = 70, 220 g) were housed individually in a room with controlled temperature (21°C) and humidity under a reversed 12L : 12D h cycle. Experiments were performed during the dark cycle (red light on) in the same room. The rats had free access to food and tap water.

Under anaesthesia (Hypnorm, 0.1 ml/100 g SC) animals were implanted with a polyethylene cannula (0.4-mm inner diameter, 0.8-mm outer diameter) into the right lateral cerebral ventricle. According to standardized procedures, the correct ICV placement of the cannula was verified (3).

Drugs

RU28318, a specific MR antagonist (MRa; Roussel Uclaf, Paris, France) was dissolved in 100% ethanol and diluted with 0.9% physiological saline to a final concentration of 100 ng/μl that contained 2% ethanol. The same dose of MRa was found to affect behavior and neuroendocrine regulation (16, 18). ACTH(4–9) (Org2766; Organon, Oss, The Netherlands) was dissolved in 0.9% physiological saline.

Immediately after ICV cannulation, and during the postoperative period, animals received a SC injection of ACTH(4–9) (1 μg in 0.5 ml per rat SC) or saline every 48 h (in total 6 injections). Rats of these two treatment groups were randomly distributed in groups receiving intracerebroventricular injections of RU28318 (100 ng in 1 μl ICV) or the diluent vehicle (1 μl): sal/veh; sal/MRa; ACTH/MRa; ACTH/veh. Groups consisted of 8 to 18 rats. To prevent a possible acute action of ACTH(4–9), the water maze was run 48 h after the last injection of the peptide. One week after the water maze the inhibitory avoidance task was run using the same animals.

Morris Water Maze

The water maze was a circular pool (110-cm diameter, 50-cm high) that was filled with water (30-cm depth, 26 ± 1°C) made opaque with white chalk. The pool was divided into four quadrants of equal size. An escape platform (7-cm diameter) was placed in the middle of quadrant 2, 1.5 cm below the water surface. Four different starting positions were equally spaced around the perimeter of the pool. During days 1 to 4, all four start positions were used once in a random sequence. A trial began by placing the rat into the water facing the wall of the pool at one of the starting points. It was given a maximum of 120 s to find the platform and was allowed to stay on it for 30 s. If the rat failed to escape within 120 s, then it was placed onto the platform for 30 s. Escape latency and distance swam were automatically registered with a video-computersystem developed by Spruijt et al.(24). On day 5 the rats performed one trial without the platform, lasting 1 min (free-swim trial). Every day rats were injected with RU28318 or vehicle 60 min before the trials.

Step Through Avoidance Task

A small elevated and illuminated platform was attached to a dark compartment with a metal grid floor (1). During three baseline trials the rat was placed onto the platform, entered the dark compartment within a few seconds and remained there for 10 s. After entering the dark compartment on the fourth trial, the guillotine door was closed and an electric foot-shock (0.65 mA, lasting 2 s) was given. A test trial was run 24 h later. The time the animal needed to enter the dark compartment (latency) was registered with a cut-off latency of 300 s. Rats received an injection of RU28318 or vehicle either 60 min before the shock (preshock) or 60 min before the test (pretest).

Statistical Analysis

Analysis of variance (ANOVA) on two factors (pretreatment with the peptide, treatment with MR antagonist) with repeated measurements were used to analyze the latency and distance swam to reach the platform during 4 days. The length of the covered distance per quadrant was expressed as percentage of the total distance. For comparing the length of the swimming path in the previously reinforced quadrant 2 with the other quadrants (1, 3 and 4), a Friedman analysis was used. The means of the groups in quadrant 2 were compared by Kruskal–Wallis test followed by Mann–Whitney U-test. These two tests were also used for evaluating the latency of passive avoidance behavior. All calculations were performed using the statistical package SYSTAT (Wilkinson, Leland, SYSTAT: The System for Statistics. Evanston, IL: SYSTAT, Inc. 1988).

RESULTS

Time and distance swam to find the platform were comparable for the four groups on all 4 days (data not shown). No significant effects of either the ACTH(4–9) analog or RU28318 could be assessed on the acquisition of the task. Figure 1 shows the length of the swim path as percentage swam in the quadrants during the free-swim trial. All groups swam more in quadrant 2 than in the other quadrants (Friedman $p < 0.001$; significant difference between groups for quadrant 2: Kruskal–Wallis 11.867, $p < 0.01$). The saline group treated with RU28318 (sal/MRa) swam a significantly shorter path in quadrant 2 than the other groups ($p < 0.05$).

Before shock, rats entered the dark compartment in less than 15 s. As Fig. 2 shows, groups differed in their test step-through latencies, if they were treated 1 h preshock (KW =

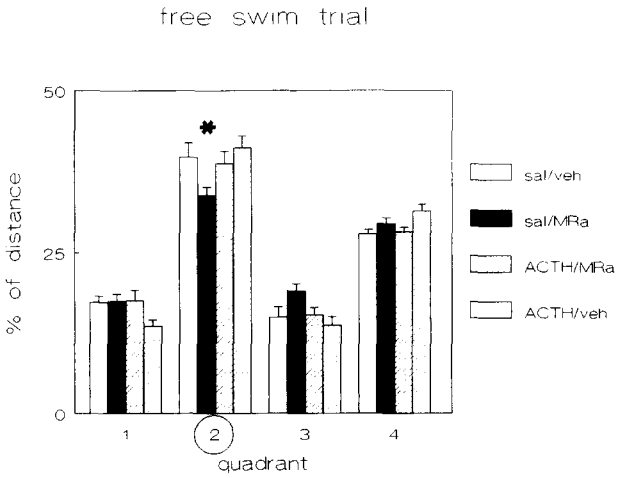

FIG. 1. Percent (mean + SEM) of the length of the swimming path swum in the four quadrants of the pool during the free-swim trial. The platform had been removed from quadrant 2 (encircled). Rats were pretreated with the ACTH(4–9) analog (ACTH) or saline (sal) and had received an ICV injection of RU28318 (MRa: 100 ng/μl) or vehicle (veh; 1 μl) 60 min before the behavioral test. *$p < 0.05$.

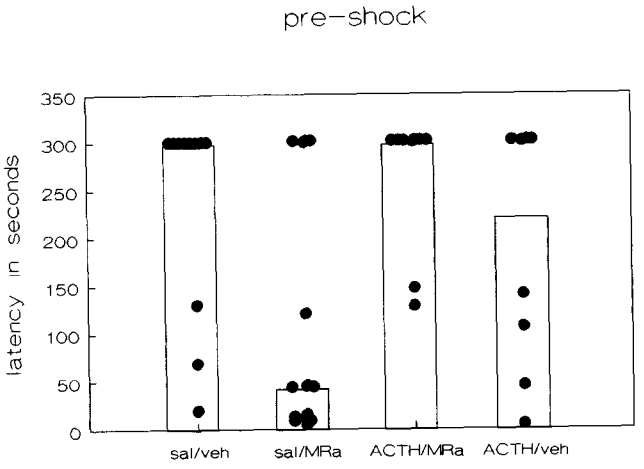

FIG. 2. Step-through avoidance test: individual (filled circles) and median (columns) step-through latencies of rats that were injected ICV 60 min before the shock. Abbreviations: see text.

9.150; $p < 0.01$). RU28318 significantly decreased the step-through latencies in saline pretreated rats (sal/MRa vs. other groups: $p < 0.02$). Pretreatment with the ACTH(4-9) analog counteracted the decrease in step-through latencies in RU28318 injected rats. Pretest injected rats did not differ in their step-through latencies (KW = 1.671, $p < 0.643$).

DISCUSSION

The results of this study demonstrate that the blockade of corticosteroid effects mediated via MR alter aspects of spatial and inhibitory avoidance learning. During the free-swim trial in the water maze, rats treated with the MRa RU28318 searched less in the platform quadrant, whereas the acquisition of the task itself was not affected. Only the preshock, but not the pretest, injection of the MRa changed inhibitory avoidance behavior. ACTH(4-9) pretreatment protected the rat against the alteration of the behavioral responses due to MRa treatment on spatial and inhibitory avoidance learning.

That the blockade of the MRs altered search strategies confirms previous results (16). Under a different testing schedule rats were injected ICV only once with 100 ng of a MRa at specified times of spatial navigation learning. Only if training and free-swim trial were run on the same day, the pretraining treatment of MRa altered the search pattern of rats in the absence of the platform, without influencing the time needed to find the platform in the training trials. Additionally, MRa treatment also influenced the swim pattern of rats under conditions where a platform never had been available. It was concluded that the blockade of MR results in an altered evaluation of an aversive environment (16). The same could hold true for the effect of MRa on inhibitory avoidance behavior. These findings support the role of MR-mediated corticosteroid effects in setting the threshold or sensitivity of the stress response and accordingly adequate behavioral strategies (6,16). As electrophysiological studies on hippocampal CA1 neurons showed (12), MRs and GRs coordinately and differentially mediate the corticosteroid control of ion regulation and transmitter responsiveness whereby MRs are involved in the maintenance of neuronal excitability. Apparently, the altered behavioral response found after MR blockade is due to a decreased level of neuronal excitation.

The balance in hippocampal excitation and inhibition is critical for attention and information processing (4). The neuropeptide ACTH has been associated with enhanced and sustained attention in a variety of studies [for review see (2)]. The maintenance of an appropriate level of hippocampal activity, as seen in aging studies on rats, appears to be related to MR activity (28). However, it is not clear if ACTH interacts directly or indirectly with MRs. Recently, an interaction between the ACTH analog and NMDA-receptor activity has been demonstrated (23,27). The known convulsant and anticonvulsant activity of ACTH-like peptides (5,26,27,29) suggests a complex, but significant interaction between ACTH and excitatory processes of the hippocampus. Most important is that intact animals in all the above mentioned studies are not influenced by treatment with an ACTH analog. Interestingly, animals kept in isolation show a number of behavioral changes among others an increase in social interest, which can be counteracted by ACTH(4-9) as well (7). Only rats which are challenged (by aging, lesions, treatment with convulsant agents, or housing conditions) seem susceptible to peptide treatment resulting into a restoration of the equilibrium between excitation and inhibition. Such restoration may underlie either compensatory processes and/or limit the consequences of the damage.

It is assumed that peptide enhanced excitation promotes compensatory processes (11,13) and that this may account for the results of the FF-lesion studies in which enhanced behavioral recovery was seen 3 weeks after the damage. In the peripheral nervous system, functional recovery after chronic ACTH(4-9) treatment was due to facilitated axonal outgrowth (10). Because reinnervation of the hippocampus after a FF lesion requires months rather than weeks (8,9), the hypothesis that ACTH(4-9) modulates hippocampal functioning, which facilitates its ability to recover, remains plausible.

Further studies on the effects of the ACTH(4-9) analog on the recovery of learning abilities after hippocampal damage will be focused on the modulation of neurotransmitters such as the excitatory amino acids which are involved in neuronal damage, neuronal excitation, and learning and memory.

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