PHARMACOLOGY AND TOXICOLOGY

Effect of Dehydroepiandrosterone on Avoidance Behavior of Adult Male Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 63-67, July, 2004
Original article submitted November 22, 2002

We studied the effect of repeated intraperitoneal treatment with dehydroepiandrosterone in doses of 0.1 and 0.7 mg/kg on conditioned-response activity and behavior of adult male rats. The effect of dehydroepiandrosterone on learning was estimated in conditioned active and passive avoidance response paradigms. Chronic administration of dehydroepiandrosterone in low and high doses had no effect on retention of conditioned passive avoidance response in adult male rats 24 h after learning. However, chronic administration of dehydroepiandrosterone in low dose impaired acquisition of the conditioned active avoidance response. It should be emphasized that chronic administration of dehydroepiandrosterone in high dose did not modulate acquisition and retention of this reaction.

Key Words: *dehydroepiandrosterone; learning; memory; male rats*

Dehydroepiandrosterone (DHEA) and its sulfate derivative are synthesized in the adrenal cortex and serve as precursors for sex steroids (androgens and estrogens). The synthesis of these substances is determined by age [3,4]. Worsening of the general state is paralleled by age-related decrease in DHEA secretion. DHEA acts as a neurosteroid hormone and is synthesized by neurons and glial cells in the central nervous system [4,5].

DHEA produces a variety of complex behavioral reactions. For example, DHEA improves cognitive function in old rats (1-1.5 years), modulates learning and memory in young mice (1-2 months) [7,8,11,15], and affects locomotor activity [9], emotionality, and anxiety [9,12,13]. The effects of DHEA on behavioral characteristics of adult male rats remain unknown.

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The effect of repeated treatment with DHEA on conditioned-response activity in adult male rats was studied on various models of learning.

MATERIALS AND METHODS

Experiments were performed on 120 adult male outbred albino rats weighing 180-200 g and obtained from the Rappolovo nursery. The animals were kept in a vivarium under natural light/dark regimen, standard temperature and feeding conditions and had free access to water and food. The study was conducted at 9.00-12.00. Behavioral tests were performed with 3 groups of animals (10 rats each). The control group included intact males treated with physiological saline. Intact animals receiving intraperitoneal injections of DHEA in doses of 0.1 and 0.7 mg/kg entered groups 1 and 2, respectively. DHEA was administered for 10 days before the start of behavioral tests and then daily during learning and testing.

The effect of DHEA on learning capacity was determined in tests for conditioned active avoidance

0007-4888/04/1381-0054

response (CAAR) and conditioned passive avoidance response (CPAR) [1]. The behavior of animals was assessed in the open field [2]. The degree of anxiety was evaluated in an elevated plus-maze [14].

CAAR was elicited in a Y-maze consisting of 3 compartments [1]. The rats were adapted to the chamber for 5 min. Turning the light on in one of the three compartments (safe compartment) for 10 sec served as a conditioned stimulus. If the rat did not enter the safe compartment, electric current (50 Hz, 10 msec) was delivered through an electrode floor of other compartments, which caused the rat to transit into the illuminated compartment. Current thresholds were estimated by vocalization (2.0-4.0 mA). The animals remained in the light compartment for 30 sec. Learning was performed under constant reinforcement. Conditioned (light) and unconditioned stimuli (electric current) were presented daily in combination (10 presentations). The correct response suggested transition of the rat into the safe compartment before electric stimulation. The training session continued for 7 days (*i.e.*, CAAR was elicited on days 1-7). Extinction of CAAR was tested after 24 h (days 8-12). The rat was placed in the maze. The same number of conditioned stimuli was delivered without electric current reinforcement. CAAR was considered to be retained when the rat exhibited even one correct response. Testing was conducted over 5 days.

CPAR was trained in a two-chamber device with single electrocutaneous reinforcement. The device was divided into large illuminated chamber and small dark chamber with electrified floor. The chambers were communicated with each other via a round hole [1]. The methodical peculiarity of our study is that CPAR was elicited by high electric current (6 mA). It should be emphasized that the animals did not exhibit severe convulsions. The rat was placed in a central area of the illuminated chamber. The tail was directed toward the hole into the dark chamber. The animal explored the chamber, found the communicating hole, and entered the dark chamber. We recorded the latency of transition into the dark chamber. Electric current (50 Hz, 6 mA) was delivered through the electrode floor of the dark chamber by the end of the 3rd minute. This treatment caused the rat to transit into the light chamber. Then CPAR was considered to be elicited. The results of training were analyzed after 24 h. The rat was placed in the setup for 3 min. We recorded the latency of transition into the dark chamber. Disregard of the dark chamber was considered as passive avoidance performance. Shortening of the time spent in the illuminated chamber reflected amnesia.

Behavioral characteristics of rats in the open field were studied by the method of E. S. Petrov *et al.* with modifications [2]. The open field was an illuminated area (80×80 cm) surrounded by walls (36 cm) and divided into 16 equal squares (19.5×19.5 cm). The animal was placed in a central area of the open field for 3 min. Horizontal (ambulations) and vertical locomotor activity (rearing postures), exploratory activity (exploration of holes), grooming behavior (scratching, licking, washing, *etc.*), and emotional reactions (defecation and urination) were recorded automatically on a hardware-software open-field device ("Biological Device", D. I. Ul'yanov State Electrotechnical University).

The degree of anxiety was evaluated in a plus maze. The maze was elevated by 50 cm above the floor and had 2 closed (with walls) and 2 open arms (without walls). The length and width of the arms were 45 and 10 cm, respectively. The height of walls in the closed arms was 10 cm [14]. A 10×10 cm open area was positioned in the center. The number of entries and the time spent in the open arms were recorded for 5 min.

The results were analyzed by Student's *t* test (Statgraphics software).

RESULTS

DHEA in a dose of 0.1 mg/kg impaired CAAR learning in male rats (Fig. 1). The animals did not exhibit CAAR over all period of training. We observed only escape reaction, which was manifested in transition into the safe compartment after electric stimulation.

Male rats receiving 0.7 mg/kg DHEA exhibited a correct response to the conditioned stimulus on day 2 of training (similarly to control animals). On day 5 the number of CAAR in these animals reached the level

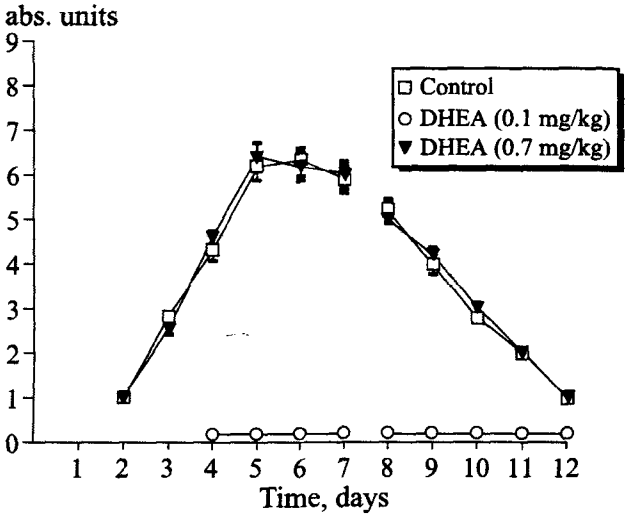

Fig. 1. Effect of chronic treatment with dehydroepiandrosterone (DHEA) on acquisition and extinction of conditioned active avoidance response (CAAR) in adult male rats. Ordinate: number of correct runs.

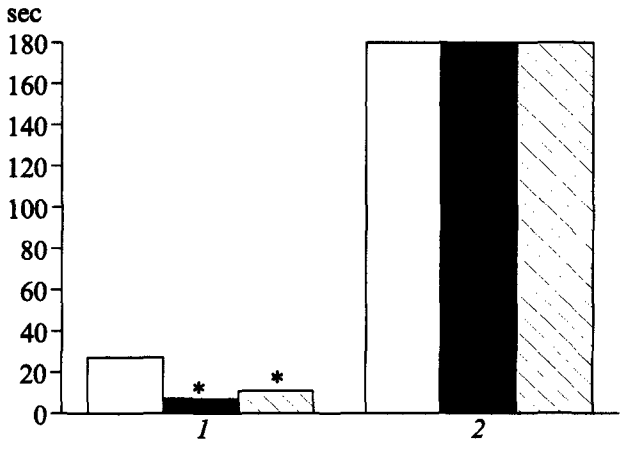

Fig. 2. Effect of chronic treatment with DHEA on the CPAR formation and performance in adult male rats. Ordinate: latency of transition into the dark chamber (sec). Latency during training (*1*) and study of CPAR after 24 h (*2*). Here and in Fig. 3: light bars, control; dark bars, 0.1 mg/kg DHEA; shaded bars, 0.7 mg/kg DHEA. *$p<0.05$ compared to the control.

observed in trained intact rats (6.6±0.2). The number of avoidance reactions in rats of groups 1 and 2 insignificantly increased on days 6 and 7 (7.0±0.2 and 6.8±0.2, respectively).

After training session (days 8-12) CAAR performance without electric current reinforcement practically did not differ in control rats and animals receiving 0.7 mg/kg DHEA.

Chronic administration of DHEA in doses of 0.1 and 0.7 mg/kg did not modulate CPAR performance 24 h after training (compared to intact animals). However, the rats receiving DHEA were characterized by shorter latency of transition into the dark chamber on day 1 of training ($p<0.001$, Fig. 2).

Chronic administration of DHEA in a low dose of 0.1 mg/kg significantly suppressed horizontal and vertical locomotor activity and grooming behavior in the open-field test ($p<0.05$). DHEA in a high dose of 0.7 mg/kg had no effect on the behavior of male rats.

Testing in the plus-maze showed that chronic administration of DHEA in low dose was followed by freezing of rats in open arms, but did not affect the number of entries into these compartments (Fig. 3). Chronic administration of DHEA in a dose of 0.7 mg/kg significantly increased the number of entries and the time spent in the open arms of the maze ($p<0.05$).

Our results show that chronic administration of DHEA had different effects on learning in adult male rats. DHEA in a dose of 0.1 mg/kg impaired the ability of animals to learn CAAR, which was probably related to disturbances in differentiation reaction and temporal circuit, and profound changes in the balance between the inhibition and excitation processes. DHEA in a dose of 0.7 mg/kg had no effect on CAAR acquisition and retention in adult male rats and did not impair active learning.

Chronic administration of DHEA in low (0.1 mg/kg) and high doses (0.7 mg/kg) did not affect retrieval of memory traces during passive learning, but decreased the latency of transition into the dark chamber on day 1 of training. This reflects primary changes in the type of biological behavior (preference of the dark compartment). The effects of DHEA in low dose on CAAR and CPAR can be associated with differences in the functional organization of active and passive avoidance in rats.

The model of CAAR characterizes the ability of animals not only to gain and retain a qualitatively new adaptive response associated with the active component of behavior, but also to perform the reaction of active avoidance [10]. Nociceptive stimulation induces the feeling of fear, which is accompanied by the discrete adaptive reaction for escape and avoidance. The avoidance reaction reflects the closure of a temporal conditioned-response relationship between conditio-

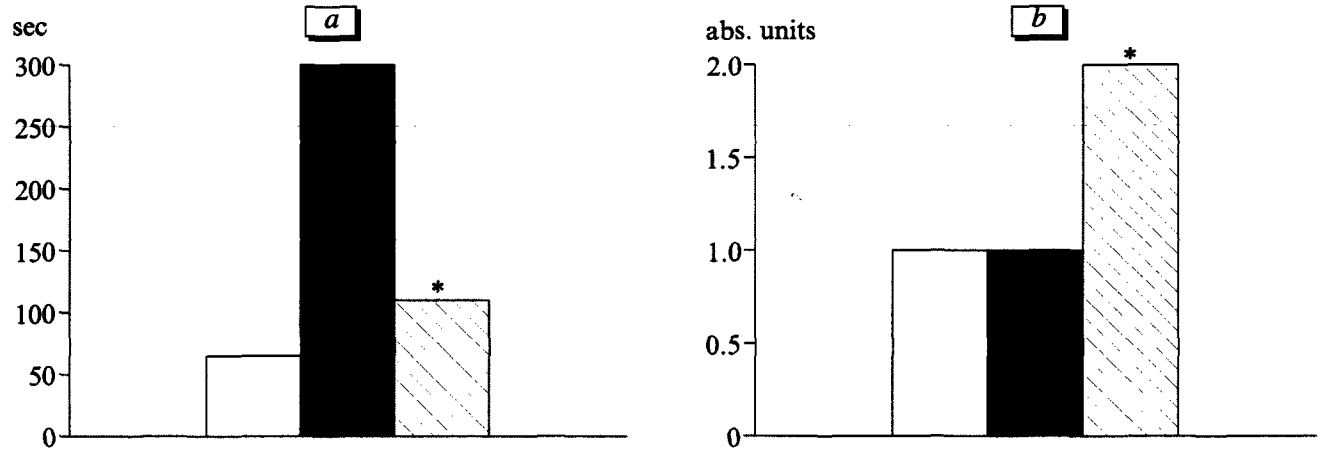

Fig. 3. Effect of chronic treatment with DHEA on the behavior of adult male rats in elevated plus-maze. Time spent (sec, *a*) and number of entries into the open arms (*b*).

ned and unconditioned stimuli. The absence of a dangerous or safe compartment suggests the formation of a differential reaction determined by the overall balance between inhibition and excitation [10].

In our study the animals were trained to escape adverse stimulation via suppression of the specific biological behavior [1,10]. The existence of a dangerous compartment (dark chamber) contributed to performance of the only adaptive reaction. The animals feel fear and suppress the desire to enter the dark chamber, which is related to the prevalence of inhibition [10]. Experiments with CPAR revealed the prevalence of inhibition in the brain of rats receiving 0.1 mg/kg DHEA. Passive avoidance learning is associated with suppression of natural biological behavior.

DHEA in low dose impaired conditioned-response activity, which probably results from changes in the goal-directed behavior of animals. The decrease in locomotor activity in the open field and freezing in the elevated plus-maze correlated with the impairment of active learning. By contrast, chronic administration of DHEA in high dose produced an anxiolytic effect on rats in the plus-maze.

Published data show that subcutaneous injection of DHEA sulfate in a single dose of 0.125-10 mg/kg improves CPAR retention in old mice (1 years) [7,8, 15]. J. F. Flood and E. Roberts demonstrated that chronic administration of DHEA sulfate (1.0-1.5 mg/kg subcutaneously) promotes CAAR performance in middle-age (18 months) and old mice (24 months), but not in young animals (1.5-2 months) [7]. J. Flood *et al.* found that administration of DHEA sulfate in a dose of 700 μg for 2 months inhibits CAAR extinction in poor learners [8]. Some authors demonstrated that the influence of DHEA on cognitive function depends on the age of animals and model of learning [8,9,11,15]. The effects of various nonspecific factors, including locomotor activity and emotionality, should be taken into account in the study of learning. DHEA sulfate reduced locomotor activity in the open field and modulated the degree of anxiety in the elevated plus-maze and Porsolt test [9,12,13].

Our results suggest that chronic administration of DHEA in low and high doses has no effect on CPAR retention in adult male rats 24 h after training. However, chronic administration of DHEA in low dose impairs CAAR acquisition. Chronic administration of DHEA in high dose does not modulate acquisition and retention of active avoidance behavior.

This work was supported by the Regional Social Foundation for Russian Medicine.

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