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Selective Effects of Dehydroepiandrosterone Sulfate on Corticoliberin-Induced Anxiety

O. G. Semenova, V. V. Rakitskaya, E. A. Vershinina, N. E. Ordyan, and V. G. Shalyapina*

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The effects of dehydroepiandrosterone sulfate (DHEA-S) on changes in the levels of anxiety induced by administration of the stress neurohormone corticoliberin were studied. A T maze was used to select Wistar rats with active and passive strategies of adaptive behavior. Testing of the active group in an elevated plus maze was used to select low- and high-anxiety individuals. Intranasal administration of corticoliberin to low-anxiety active rats resulted in decreases in total activity and increases in the level of anxiety, while high-anxiety animals had low sensitivity to this neurohormone. Prior administration of DHEA-S at a dose of 3 mg/100 g had antistress effects in low-anxiety rats and anxiolytic effects in high-anxiety rats. In passive animals, which were characterized by initially high levels of anxiety and were resistant to corticoliberin, administration of DHEA-S also had antistress actions. These results led to the conclusion that the effects of DHEA-S depended on the initial psychoemotional state and behavioral sensitivity to corticoliberin.

Keywords: corticoliberin, dehydroepiandrosterone sulfate, anxiety, rats, behavior.

The therapeutic properties of dehydroepiandrosterone (DHEA), a steroid hormone of the reticular zone of the adrenal cortex, has been known since the 1990s [9], though it has only recently entered wide use in clinical practice [20, 24]. Its wide introduction has resulted from extensive data on its pharmacological efficacy in various somatic [2] and especially mental illnesses with impairment of attention and memory, cognitive activity, and age-related dementia [1, 15]. The greatest interest in recent years, however, has been in studies on the successful use of DHEA in the treatment of depression, including post-traumatic stress disorder occurring after extreme shock or severe unavoidable situations [26]. The sulfate of the hormone (DHEA-S) is characterized by a high level of potentiation due to increased hydrophilicity and preferential interaction with GABA receptors [18]. We have suggested that these hormones may have stress-protective actions and that their prior administration can soothe the symptoms of stress and prevent the development of post-stress pathology.

The anxiolytic properties of DHEA and DHEA-S have been demonstrated in experiments on mice and rats using a variety of behavioral tests [14, 19]. However, there are also data showing that these hormones induce effects of different strengths in animals with different levels of anxiety: the clear anxiogenic effects of DHEA-S on behavior are seen only in individuals with increased anxiety [3, 22], which suggests individual sensitivity to the actions of this steroid due to differences in the neurotransmitter and other components of the organization of behavioral reactions. One such component may consist of the corticoliberinergic system of the brain, which is a triggering element for stress and integrates endocrine functions and behavior during the development of stress [11]. Corticoliberin serves as a neurochemical factor in the development of situational and reactive anxiety which is an obligatory component of any stress response. During stress reactions, this action of corticoliberin undoubtedly has adaptive value, as the body's defensive forces are mobilized during the "anxiety phase" and the

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, 6 Makarov Bank, 199034 St. Petersburg, Russia; e-mail@ vera@infran.ru.

* Deceased.

TABLE 1. Behavior of Active and Passive Rats in a T Maze

Parameter	Active (n = 93)	Passive (n = 37)
Index of behavioral activity, %	90.8 ± 1.5	58.2 ± 4.0*
Index of behavioral passivity, %	1.8 ± 0.4	35.7 ± 4.5*
Latent period, sec	8.7 ± 0.8	38.9 ± 5.6*
Number of squares crossed, n	130.5 ± 4.0	41.2 ± 4.0*
Freezing time, sec	0.5 ± 0.3	29.3 ± 9.0*
Grooming time, sec	7.7 ± 1.6	9.7 ± 2.1
Number of boluses, n	0.1 ± 0.05	1.3 ± 0.6*
Number of rearings, n	21.5 ± 0.9	9.9 ± 1.3*
Number of turns, n	0.37 ± 0.08	1.4 ± 0.2*

Note. *Significant ($p < 0.05$) differences between active and passive animals.

corresponding strategy of adaptive behavior is formed. However, in the case of severe or chronic stress, the corticoliberinergic system is hyperactivated, which may result in the development of pathological anxiety and subsequent post-traumatic depression [7].

Our studies have shown that rats with an active behavioral strategy have high sensitivity to corticoliberin and are regarded as reactive to this agent. Administration of corticoliberin to rats with a passive strategy of adaptive behavior produces no visible effect, as they have high initial levels of anxiety, apparent as a behavioral deficit [5, 6]. Repeated administration of corticoliberin to these animals, however, leads to the development of marked depression, and exposure of these animals to unavoidable stress increases the development of post-stress psychopathology [4].

As noted above, DHEA and DHEA-S induce different effects in animals with high and low levels of anxiety. It can therefore be suggested that the anxiolytic effects of these hormones should be different in animals with different strategies of adaptive behavior. Thus, the aim of the present work was to investigate the effects of prior doses of dehydroepiandrosterone sulfate on anxiety induced by corticoliberin in rats with different behavioral strategies.

METHODS

Experiments were performed using adult male Wistar rats weighing 250–280 g observing ethical regulations for studies involving animals laid out in European Communities Council Direction 86/609 EEC. Animals were kept in plastic cages in groups of six on a standard diet with free access to water and food.

Strategies of adaptive behavior were assessed using a T maze as described previously [6]. The results yielded overall assessments of the animals' behavior. The indexes of behavioral activity (IBA) and passivity (IBP) were calculated for each individual animal. Behavioral activity included all forms of vertical and horizontal activity, excluding grooming, and IBA was calculated as the proportion of time spent by the animal running, rearing, and moving on the spot (except grooming) as a percentage of the total observation time (3 min). Behavioral passivity included freezing and sitting. IBP was calculated as the proportion of time during which the animal was immobile (the latent period of entry into the maze arm, freezing, and sitting) as a percentage of the total test duration. As the distributions of these indexes were sharply asymmetrical, logarithms were taken of the data and these were used to define the group boundaries. Group boundaries consisted of the 25% quantiles of the mean, and the distribution into parts was as follows: $X_1 = M - 0.67SD$, $X_2 = M \pm SD$, $X_3 = M + 0.67SD$, where M is the mean, and SD is the mean square deviation (standard deviation). These indexes were used to select, from all the animals tested, those animals with high (IBA 85–100%, IBP 0–4.6%) and low (IBA 0–70%, IBP 15–100%) activity. The behavioral characteristics of active and passive animals are shown in Table 1.

Furthermore, the T maze was used to calculate the number of turns in the maze arms as a behavioral characteristic. Turns were regarded as changes in the rat's movement direction at incomplete penetration of the maze arm. We believe that this measure provides an additional indicator of the emotional component of behavior.

Initial anxiety levels were assessed in active and passive rats by testing all animals in an elevated plus maze, in which the time spent by the animals in the open arms of the maze was measured over 5 min, along with the number of episodes of hanging down, and the numbers of excursions into the open arms of the maze. These measures are inversely proportional to the level of anxiety [21, 23]. Furthermore, measures of total activity were determined, i.e., the number of vertical rearings, the number of crossings through the center of the maze, and the number of glances into the open arms of the maze, as well as the duration of grooming reactions. The results of this testing showed that active rats could be divided into groups of animals with low and high levels of anxiety (Table 2). Considering published data

TABLE 2. Behavior of Active and Passive Rats in the Elevated Plus Maze

Parameter	Active calm (n = 59)	Active anxious (n = 34)	Passive (n = 37)
Time in light arms, sec	54.8 ± 3.3	10.2 ± 2.0*	27.1 ± 5.2*
Number of excursions into light arms, n	5.0 ± 0.4	1.3 ± 0.4*	3.1 ± 0.8
Number of rearings, n	17.5 ± 0.9	11.3 ± 1.2*	15.4 ± 1.2
Number of hangings, n	6.3 ± 0.8	0.7 ± 0.2*	2.3 ± 0.8*
Number of glances, n	12.1 ± 0.5	6.5 ± 0.7*	7.1 ± 0.9*
Grooming time, sec	10.0 ± 2.1	37.3 ± 6.8*	16.0 ± 5.0
Number of crossings of center of maze, n	5.2 ± 0.4	1.6 ± 0.4*	2.4 ± 0.9*
Number of boluses, n	0	0.7 ± 0.4	0.8 ± 0.4

Note. Significant ($p < 0.05$) differences from active calm animals.

TABLE 3. Behavior of Active and Passive Rats in a T Maze in Relation to Initial Levels of Anxiety

Parameter	Active calm (n = 59)	Active anxious (n = 34)	Passive (n = 37)
Index of behavioral activity, %	90.8 ± 1.5	86.2 ± 1.9	58.2 ± 4.0* #
Index of behavioral passivity, %	1.8 ± 0.4	1.2 ± 0.6	35.7 ± 4.5* #
Latent period, sec	8.7 ± 0.8	6.7 ± 0.8	38.9 ± 5.6* #
Number of square crossings, n	130.5 ± 4.0	130.8 ± 5.8	41.2 ± 4.0* #
Freezing time, sec	0.5 ± 0.3	1.2 ± 0.4	29.3 ± 9.0* #
Grooming time, sec	7.7 ± 1.6	13.0 ± 3.0	9.7 ± 2.1
Number of boluses, n	0.1 ± 0.05	2.5 ± 0.3*	1.3 ± 0.6*
Number of rearings, n	21.5 ± 0.9	23.4 ± 1.5	9.9 ± 1.3* #
Number of turns, n	0.37 ± 0.08	1.9 ± 0.2*	1.4 ± 0.2*

Note. *Significant differences ($p < 0.05$) from active calm animals; #significant differences ($p < 0.05$) between passive and active anxious animals.

showing that the effects of DHEA-S in anxious and non-anxious individuals differ [22], experimental and control groups were formed with consideration of their initial levels of anxiety.

Active and passive animals were then divided into four groups. Animals of group 1 were given intranasal corticoliberin (Serva) at a dose of 0.5 μg in each nostril in a volume of 5 μl using the protocol described in [4, 5] and were tested in the elevated plus maze 5 min later, comparing the behavior of the experimental animals with the behavior of rats given intranasal physiological saline (group 2). The effects of DHEA-S (Sigma) on behavior in the elevated plus maze induced by corticoliberin were studied in the other two groups. Experimental animals received i.p. DHEA-S at a dose of 3 mg/100 g [2], followed one day later by intranasal corticoliberin. Control animals received i.p. physiological saline followed one day later by intranasal corticoliberin. Levels of anxiety in control and experimental groups in the elevated plus maze were determined 5 min after corticoliberin administration. These experiments were performed no less than 14 days after the initial testing of the rats in the elevated plus maze.

Data were analyzed by multidimensional two-factor analysis of variance (MANOVA) in which the factors were "treatment" (control, CRF, CRF + DHEA), and group (active calm, active anxious, passive) and multidimensional unifactorial analysis of variance within each group (active calm, active anxious, passive) with "treatment" (control, CRF, CRF + DHEA) as the factor. Paired comparison between treatments were performed using contrasts (equality/nonequality of variances) and post hoc comparisons in the framework of unifactorial analysis of variance. Data were analyzed in parallel using nonparametric tests – the Kruskal–Wallis test and the Mann–Whitney test. Statistically significant differences were identified at $p < 0.05$ using two-tailed tests.

RESULTS

Table 3 presents the results of testing the rats in a T maze with separation of the active rats into anxious and non-anxious (calm) animals. This shows that both anxious and calm active rats had higher IBA and lower IBP than

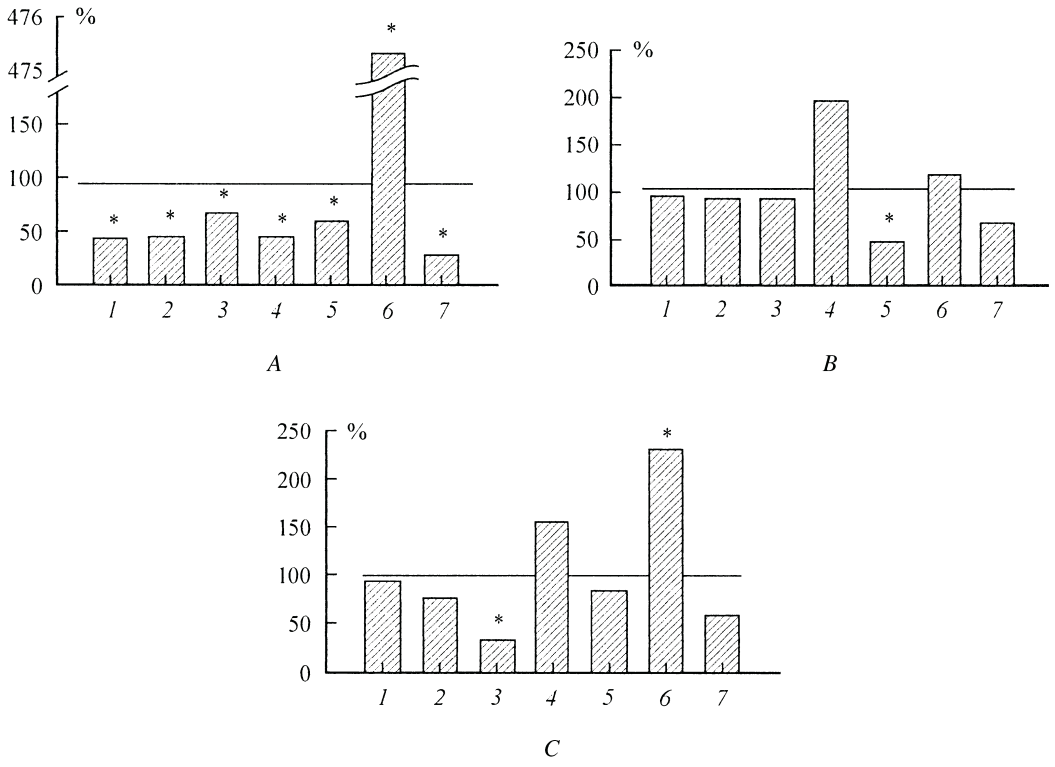

Fig. 1. Effects of corticoliberin on the behavior of rats in an elevated plus maze (control = 100%). *A*) Active calm rats; *B*) active anxious rats; *C*) passive rats. On the horizontal axes: *1*) time spent in the light arms of the maze, sec; *2*) number of excursions into the light arms, *n*; *3*) number of rearings, *n*; *4*) number of hangings *n*; *5*) number of glances, *n*; *6*) duration of grooming, sec; *7*) number of crossings through the center of the maze, *n*. *Significant differences ($p < 0.05$) compared with control animals.

passive animals. The measures on which these indexes were based (latent period of entry into the maze, number of square crossings, and number of rearings) showed corresponding significant changes. Active anxious rats differed from active calm animals in terms of the number of boluses and the number of turns, which supports our view that incomplete excursions into the maze (turns) reflect the animals' levels of emotional tension.

It should be noted that in the elevated plus maze test, active calm and active anxious rats demonstrated significant differences not only in measures of the level of anxiety (time spent in the open arms, number of excursions to the open arms, and number of episodes of hanging from them), but also in terms of measures of total activity (Table 2). Animals with higher anxiety levels performed fewer rearings and passages across the center of the maze and were characterized by longer periods of grooming. These differences in the behavior of active anxious rats in the T maze and the elevated plus maze appear to be associated with the greater stress-inducing nature of the latter.

Intranasal corticoliberin had different effects on behavior in the elevated plus maze in the different groups of rats. In active calm animals, as compared with control rats, there was a sharp reduction in total activity and an increase in the level of anxiety, which were apparent as significant reductions in virtually all the study measures except grooming reactions, whose duration increased significantly (Fig. 1, *A*). Corticoliberin had virtually no behavioral effects in active anxious rats (Fig. 1, *B*). The only effects of corticoliberin on the behavior of passive animals were in relation to the number of rearings, which decreased, and the duration of grooming, which increased significantly in these rats as compared with control rats given physiological saline (Fig. 1, *C*).

Prior administration of DHEA-S to active calm rats relieved the stress-inducing effects of corticoliberin on their behavior. This was mainly apparent in the normalization of measures of total activity, and the number of crossings through the center of the maze even increased, which is evidence of an increase in motor activity (Fig. 2, *A*). Judging from the increase in the duration of time spent by the rats in the open arms of the maze, anxiety levels in these animals decreased in response to DHEA-S. The anxiolytic effects of the hormone were more pronounced in active anxious rats, (Fig. 2, *B*). The time spent by these animals in the open arms of the maze, the number of episodes of hanging from the open arms, and the number of glances increased severalfold. At the same time, motor activity in this group of rats, which was high in the T maze and sharply reduced in the ele-

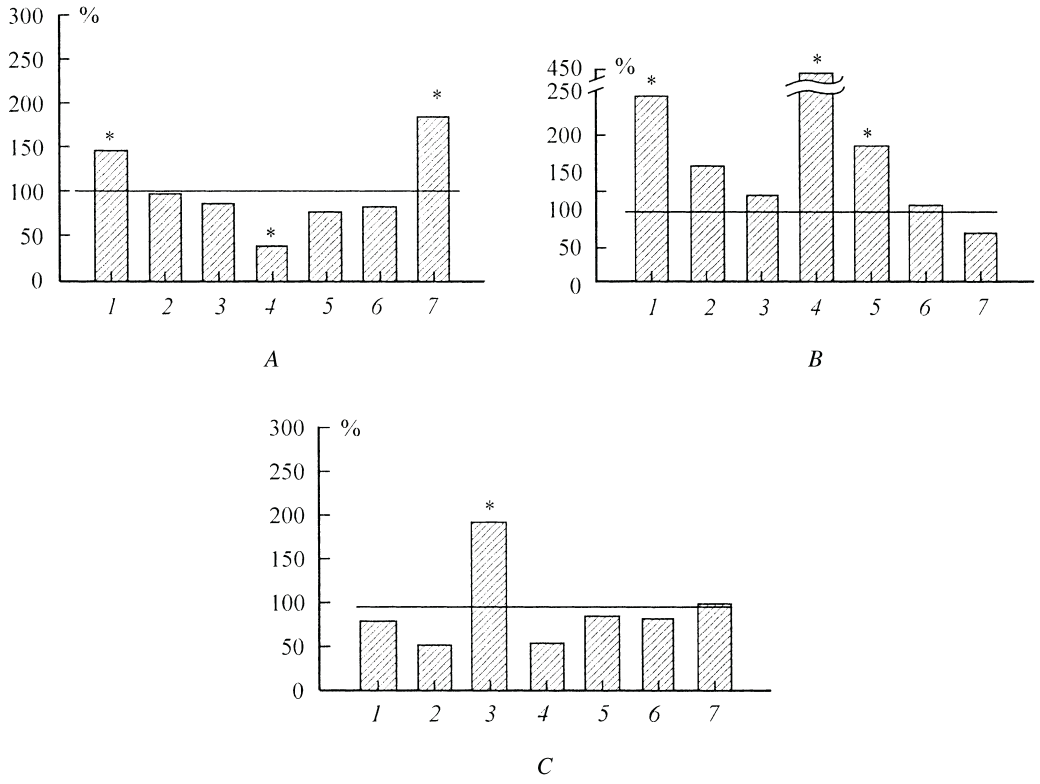

Fig. 2. Effects of prior administration of dehydroepiandrosterone sulfate on the corticotropin-induced behavior of rats in the elevated plus maze (corticoliberin only = 100%). For further details see caption to Fig. 1.

vated plus maze, did not increase after DHEA-S, which is additional support for the anxiolytic action of this steroid.

Despite the fact that corticoliberin had no significant effect on the behavior of passive rats, DHEA-S also had no antistress action in these animals. Prior administration of hormone normalized grooming duration and led to an increase in the number of rearings, thus neutralizing the effects of corticoliberin (Fig. 2, *C*).

Thus, these data lead to the conclusion that the effect of DHEA-S depends on the individual characteristics of behavioral stress reactivity and corticoliberin sensitivity.

DISCUSSION

DHEA and its sulfate, as the main precursors of the androgenic steroid hormones of the adrenal cortex, were long regarded as important regulators of sexual functions, especially pregnancy and delivery. Their relevance to adaptive processes, in contrast to corticosteroids, failed to attract attention for a prolonged period, and it is only in the last decade that this has been subjected to detailed study by physiologists and clinicians. This occurred because of the observation of high levels of these hormones in the brain; they were termed neurosteroids not only because they can be synthesized in neural tissue, but also because they have active influences on all brain functions [20, 24]. Studies in mice demonstrated the anxiolytic and antidepressant effects of DHEA and DHEA-S [16], while anxious-depressive disorders in patients are accompanied by decreases in CSF and plasma concentrations of these steroids [17].

In our studies, stress was modeled by intranasal administration of corticoliberin and the occurrence and development of the anxious state was assessed using an elevated plus maze. The combined use of a T maze and a plus maze allows assessment of movement on the one hand and, on the other, the emotional components of the animals' adaptive behavior in novel conditions. Our previous experiments using a T maze showed that active rats are characterized by high corticoliberin sensitivity, while passive animals are essentially insensitive [5, 8].

In the present studies, the elevated plus maze also demonstrated differences in the sensitivities of active and passive rats to corticoliberin – neurohormone effects in active individuals depended on the initial level of anxiety. Thus, the effects of corticoliberin in active calm rats consisted of a decrease in total activity, an increase in anxiety, and an increase in grooming duration, which can be interpreted as behavioral signs of a stress reaction. However, corticoliberin produced no significant changes in the behav-

ior of active anxious rats. In passive animals, the effects of corticoliberin on behavior were insignificant, though there was an increase in grooming time and a decrease in investigative activity apparent as a reduction in the number of rearings. Prior administration of DHEA-S completely blocked the stress-inducing action of corticoliberin on active calm rats and had a marked anxiolytic effect in active anxious animals. DHEA-S also had antistress actions in passive rats, as the duration of grooming in these animals normalized and the number of rearings could even increase.

This differential action of DHEA-S is probably due to the interaction of this group of steroids with both $GABA_A$ receptors, for which they are negative modulators, and the receptors of other neurotransmitter systems, whose activity can define the specific features of individuals with different typological behavioral characteristics. Most attention in the literature is focused on the interaction of neurosteroids with the serotoninergic system, with which the well-known anxiolytic action of serotonin receptor blockers is linked [12]. Supporting data were then obtained for a direct interaction of DHEA with sigma-1 receptors [25], through which dopamine, noradrenaline, and glutamate release is regulated. Data on the interaction of neurosteroids with NMDA glutamate receptors [10] are particularly interesting, these mediating a multitude of intracellular processes determining neuron excitability. Recent years have seen studies supporting the involvement of neurosteroids in the receptor binding of vasopressin, oxytocin, cholecystokinin, and many other transmitters and peptides [17, 24]. This leads to the conclusion that the actions of DHEA and its sulfate form may be very diverse and may affect, directly or indirectly, the whole set of transmitter systems involved in the cascade regulation of behavioral processes. These observations led to the hypothesis that imbalance may occur between the excitatory and inhibitory mechanisms forming the final pathway to the executive systems. These views led in particular to consideration of the role of DHEA in the pathogenesis of Alzheimer's disease, in which there are not only changes in GABAergic transmission, but also impairments to the interaction of neurosteroids with acetylcholine receptors [27]. We take a similar view, suggesting that this mechanism of the impaired interaction of the GABA system and the corticoliberinergic system of the hypothalamus may underlie the pathogenesis of many post-stress disorders. In particular, this may be the real cause of changes in the endocrine functions of the sexual and hypophyseal-adrenocortical systems accompanying virtually all mental disorders, especially anxious-depressive disorders [26]. The endocrine hypothalamus is the terminal pathway receiving afferents from many transmitter systems, among which the GABAergic projections are defining in the organization of reverse feedback mechanisms and timely suppression of the adenohypophysis and adrenal cortex in stress. Corticoliberin as a neurohormone regulates the adrenocortical function of the adenohypophysis, while as a neurotransmitter it determines the behavioral stress response, especially the severity of situational and reactive anxiety [13]. One of the neurochemical mechanisms of the initial differences in emotional status may consist of differences in the corticoliberin sensitivity of the brain. Thus, the question of the interaction of neurosteroids and corticoliberin is clearly one of the most important in answering the question of the individual-typological characteristics of stress reactivity, particularly in identifying the causes of various types of post-stress psychopathology.

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