Prolonged Decrease in Stress Reactivity Caused by Dehydroepiandrosterone Sulfate

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In male rats exposed to repeated stress, the decrease in stress reactivity produced by subcutaneous injection of dehydroepiandrosterone sulfate (recorded by the decrease in stress-induced concentrations of corticosterone and adrenocorticotropic hormone in blood plasma) was observed 1-6 days postinjection and involved central regulatory mechanisms.

Key Words: *dehydroepiandrosterone sulfate; stress reactivity*

The corticosterone assay showed that dehydroepiandrosterone sulfate (DHEAS) decreases stress reactivity in male rats. Corticosterone concentration in repeatedly stressed males was measured 2 days after single administration of aqueous solution of DHEAS [2]. 11-Hydroxycorticosteroid concentration in females was estimated on day 16 after repeated administration of oil solution of synthetic dehydroepiandrosterone analogue retabolil (before and 2 weeks after treatment) [2]. Oil solutions of steroid hormones produce a prolonged effect. However, the influence of aqueous solutions remains unknown. Our previous studies showed that aqueous solution of DHEAS produced a prolonged anxiolytic effect, which developed 4 and 28 h after treatment [4].

Here we studied the effect of single treatment with aqueous solution of DHEAS on stress reactivity in male rats exposed to repeated stress (19 days). The concentrations of corticosterone and adrenocorticotropic hormone (ACTH) were measured 1, 2, 3, 4, 5, and 6 days after DHEAS injection.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 160-180 g. The rats were subjected to repeated shaking (1 h/day, 19 days) on an AVB-4p laboratory shaker at a frequency of 180 rpm. Stress reactivity was determined by measuring the concentrations of corticosterone and ACTH in blood plasma immediately after stress. Plasma corticosterone was assayed by high-performance liquid chromatography on a microcolumn with anticoagulant heparin. ACTH concentration was estimated by radioimmune assay (standard kit (ELISA-ACTH) with anticoagulant EDTA using antibodies to fragment 1-24. Human ACTH is a polypeptide consisting of 39 amino acid residues. The N-terminal peptide 1-24 is similar in all vertebrate species. Biological activity of peptide 1-24 is similar to that of ACTH. Moreover, the effects of these compounds persist over the same period. Plasma DHEAS concentration was measured using standard DHEA sulfate kit.

The animals were decapitated on days 1, 2, 3, 4, 5, and 6 after injection of 30 mg/kg DHEAS sodium salt (Sigma). All experimental procedures were performed according to international rules on laboratory animal care.

The results were analyzed by means of Statistica software. The significance of differences was evaluated by Student's *t* test.

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RESULTS

The concentrations of corticosterone (Fig. 1) and ACTH (Fig. 2) in blood plasma from male rats increased immediately after repeated stress. The increase in corticosterone concentration after repeated stress (19 days) was less pronounced in animals receiving single subcutaneous injection of DHEAS 1 ($p<0.01$), 2 ($p<0.01$), 3 ($p<0.05$), 4 ($p<0.01$), 5 ($p<0.01$), and 6 days ($p<0.01$) before decapitation (compared to stressed rats without hormone treatment, Fig. 1). No differences in corticosterone concentration were revealed on days 1, 2, 3, 4, 5, and 6 after DHEAS administration.

ACTH concentration in repeatedly stressed rats decreased 1-6 days after hormone treatment (Fig. 2). The increase in ACTH concentration was less pronounced on days 2 and 6 after DHEAS injection ($p<0.05$ compared to stressed rats without hormone treatment). Moreover, ACTH concentration on days 1, 2, 3, 4, 5, and 6 after DHEAS injection did not differ from the control.

Thus, injection of DHEAS aqueous solution limits the increase in corticosterone and ACTH concentrations in response to repeated stress, which attests to a prolonged stress-limiting effect of this preparation. The prolonged effect of oil solutions of steroid hormones is associated with the influence of oil. The effect of aqueous steroid solution was unexpected.

In experiments studying the role of DHEA and DHEAS in correction of pathological changes usually long-term (from several weeks to 1 year) daily treatment with these compounds is employed [5,7], while in behavioral experiments they are administered several minutes before testing [10,11]. The results of our study and published data [3,4] suggest that daily treatment with DHEAS for a long time is not required to prevent pathological changes [5,7]. It is not necessary to administer this drug immediately before behavioral tests, since aqueous solution of DHEAS produces a prolonged effect for at least 6 days [10,11]. These data are of considerable importance for the study of stress reactivity, since injection produces a considerable stress response.

DHEAS modulates stress reactivity by affecting the concentration of ACTH. Therefore, the decrease in stress reactivity is mediated by central regulatory mechanisms with the involvement of ACTH.

A special series was conducted to measure plasma DHEAS concentration after single stress exposure (Fig. 3). Exogenous DHEAS was injected 2 h or 1 and 2 days before decapitation. Plasma DHEAS concentration increased 2 h after injection, but did not differ from the control on days 1 and 2 after

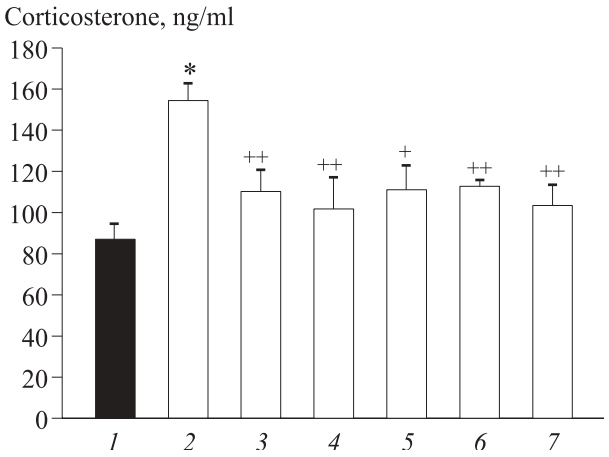

Fig. 1. Plasma corticosterone concentration in male rats after 19-day stress exposure and administration of dehydroepiandrosterone sulfate (DHEAS). Here and in Fig. 2: control (*1*); stressed animals without DHEAS administration (*2*); 1 day after DHEAS administration (*3*); 2 days after DHEAS administration (*4*); 3 days after DHEAS administration (*5*); 4 days after DHEAS administration (*6*); 6 days after DHEAS administration (*7*). *$p<0.001$ compared to the control; $^{+}p<0.05$ and $^{++}p<0.01$ compared to 19-day stress exposure without DHEAS administration.

treatment. Therefore, the prolonged effect of DHEAS is not associated with long-term presence of this compound in the circulation. The delayed effect of this hormone is realized via intermediate mechanisms.

Our previous studies showed that the anxiolytic [4] and stress-limiting effects of DHEA [3] involve the opiate system (*e.*g., μ-opioid receptors). The prolonged effect of DHEAS probably results from its interaction with the opiate system. They modulate activity of other neurotransmitter systems (monoaminergic systems), which, in turn, modulate activity of the hypothalamic-pituitary-adrenocortical system [8].

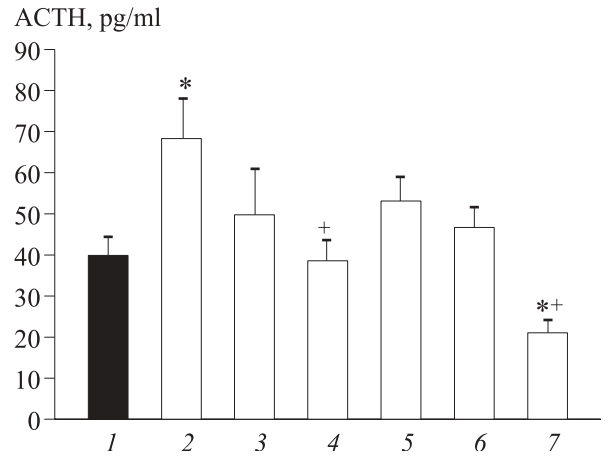

Fig. 2. Plasma ACTH concentration in male rats after 19-day stress exposure and administration of DHEAS. $p<0.05$: *compared to the control; $^{+}$compared to 19-day stress exposure without DHEAS administration.

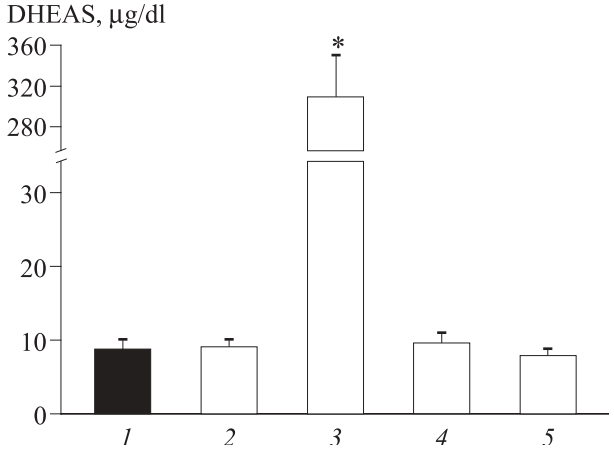

Fig. 3. Plasma DHEAS concentration in stressed male rats 2 h and 1 or 2 days after DHEAS administration. Control (*1*); after single stress (*2*); 2 h after DHEAS administration (*3*); 1 day after DHEAS administration (*4*); 2 days after DHEAS administration (*5*). **p*<0.001 compared to the control.

This hypothesis is supported by published data. Although the half-life period of peptide regulators is several minutes, their effect on physiological processes persists over hours or days [1]. Peptide hormones (*e.g.*, opioids) modulate monoaminergic transmission by various mechanisms. They affect catabolism or biosynthesis of monoamines in nerve terminals, function of postsynaptic receptors for monoamines, release and uptake of the neurotransmitter, and membrane permeability [1,9,12]. The effects of opioids on monoaminergic systems can be realized via μ-opiate receptors [6,14]. Moreover, DHEAS modulates activity of the noradrenergic and dopaminergic system [13].

We conclude that DHEAS produces a prolonged decrease in stress reactivity in animals subjected to repeated (19 days) stress. The effect persists over 1-6 days after subcutaneous injection of DHEAS. The stress-limiting effect of DHEAS is mediated by central regulatory mechanisms with the involvement of endogenous ACTH. The prolonged effect of DHEAS is not associated with long-term presence of this compound in the circulation. This effect is mediated by various physiological mechanisms, including the μ-opioidergic mechanism.

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