PHARMACOLOGY AND TOXICOLOGY

Effect of Dehydroepiandrosterone on Radioligand Binding of [^{3}H]-Testosterone by Androgen Receptors in Rat Hypothalamus

A. A. Bairamov and N. S. Sapronov

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Intramuscular injections of dehydroepiandrosterone in a dose of 0.7 mg/kg for 10 days significantly increased nuclear and cytoplasmic fractions of androgen receptors in the preoptic/anterior hypothalamic area. Presumably, the effect of the neurosteroid is mediated by 5α-reductase transformation of dehydroepiandrosterone into 5α-dehydroepiandrosterone, which initiates the synthesis of androgen receptors.

Key Words: *dehydroepiandrosterone; androgen receptors; hypothalamus; gonadectomy*

A variety of central effects of dehydroepiandrosterone (DHEA) suggests that this neurosteroid is important for many CNS regions. DHEA receptors participate as transcription factors in gene regulation [4] and regulate the rate of protein biosynthesis [10], this providing a molecular basis for a wide spectrum of their effects on neuronal functions. Through interactions with neuromediator receptors on the cell surface, DHEA modulates activities of different neuromediator systems involved in the training and memory processes and in cognitive functions [1,5]. DHEA facilitates expression of gonadotropin-releasing hormone gene, thus modulating activity of the hypothalamo-pituitary-gonadal axis [8].

In order to clear out the effect of DHEA neurosteroid on androgen reception, we measured the concentrations of cytoplasmic and nuclear androgen receptors (AR) in the hypothalamus of male rats under conditions of sex hormone deficiency.

MATERIALS AND METHODS

Experiments were carried out on intact hemigonadectomized and gonadectomized rats, which were intramuscularly injected with DHEA in doses of 0.1 and 0.7 mg/kg. The effects of single and repeated injections of DHEA were studied. The animals were sacrificed 2 h after single injection or 2 h after the last of 10 daily injections. Controls were intramuscularly injected with normal saline. After decapitation AR concentration was measured in preoptic/anterior hypothalamic area (POAH). Material from one group was pooled for each measurement. The tissue was homogenized in Clayland buffer (1.211 g Tris, 0.558 g EDTA, 1.21 g Na_2MoO_4, 0.746 g KCl, 0.285 g $MgCl_2$, and 23 mg dithiotreitol, pH 7.4), and centrifuged for 10 min at 800*g*. The precipitate was used for isolation of nuclear fraction. The supernatant was centrifuged at 105,000*g* and 4°C for 90 min for isolation of the cytosolic fraction. 1,2,6,7,-[^{3}H]-testosterone ([^{3}H]-T, specific activity 80-110 Ci/mol, Izotop) served as the label. For evaluation of AR in the hypothalamus, cytosol aliquots were incubated for 20 h at 0-4°C with single saturating concentration of [^{3}H]-T (4×10^{-9}). In order to separate the free and bound ligand fractions, the samples were incubated with 4% dextran-charcoal mixture for 10 min at 0-4°C and centrifuged for 15 min at -4°C and 4000 rpm. In order to evaluate nonspecific binding, some samples were incubated with 400-fold excess of unlabeled testosterone. The nuclei were isolated by differentiated centrifugation in 2.2 M

Department of Neuropharmacology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** alekber@mail.ru. A. A. Bairamov

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sucrose density gradient. The samples of nuclear fraction were incubated with saturating concentration of [^{3}H]-T at 32°C for 60 min. Free hormone was separated by washout in TKE buffer followed by extraction of bound steroid in 0.4 M KCl and separation of free and protein-bound steroid with 4% dextran-charcoal mixture. The suspension was incubated for 40 min at 0°C, centrifuged for 15 min at 3000*g* and the supernatant was transferred into vials with scintillation fluid. Radioactivity was measured on a Beckman L30 scintillation β-counter. The content of AR in the cytosol was evaluated per g protein and in the nuclear fraction per mg DNA. Protein content was measured by the method of Lowry, DNA content by the method of Burton.

RESULTS

Injection of DHEA caused significant changes in the content of nuclear and cytoplasmic AR in the hypothalamus of gonadectomized rats (Fig. 1). The cytosolic fraction of AR decreased and the nuclear fraction of AR in the POAH appreciably decreased 3 weeks after bilateral gonadectomy in comparison with the control. Similar changes in AR level were characteristic of hemigonadectomized rats, though the decrease was less pronounced, particularly for the cytoplasmic AR fraction. Analysis of the brain AR level and serum testosterone showed that the time course of AR content in POAH nuclei correlated with plasma testosterone in gonadectomized rats.

Our findings attest to a dose-dependent effect of DHEA on AR content in the cytosol and nuclei of POAH (Fig 1). After a 10-day course of intramuscular injections of 0.7 mg/kg DHEA to gonadectomized males the content of cytosolic and nuclear AR fractions in the hypothalamus considerably increased ($p<0.05$). After administration of low physiological dose of DHEA (0.1 mg/kg) the content of AR changed negligibly in both gonadectomized and hemigonadectomized male rats. The nuclear fraction of AR increased significantly ($p<0.05$) in hemigonadectomized rats in response to DHEA in a dose of 0.7 mg/kg, while the increase in the content of cytosolic receptors was negligible.

The increase in AR level in gonadectomized rats after single injection of DHEA in similar doses was less pronounced than after 10 injections. The nuclear fraction of AR increased negligibly, while the increase in cytosolic AR fraction after injection of 0.7 mg/kg DHEA was more pronounced (from 140.3±12.5 to 198.5±14.2 fmol/mg protein) than after 10 injections of the hormone in the same dose (from 156.7±11.3 to 176.4±10.5 fmol/mg protein). Presumably, this increase was due to the release of the hormone molecules from cytosolic receptors under conditions of hormone deficiency after gonadectomy. Single injection of DHEA in a dose of 0.1 mg/kg had virtually no effect on the number of nuclear and cytoplasmic AR in rat hypothalamus both after uni- and bilateral gonadectomy.

The increase in AR level in POAH could be due to changed hormonal status after treatment with DHEA, that is, the 5α-reductase pathway of DHEA transformation into 5α-dehydrotestosterone initiating AR synthesis could be activated. Animal experiments showed that intravenous injections of labeled DHEA led to the appearance of labeled testosterone in the blood [3].

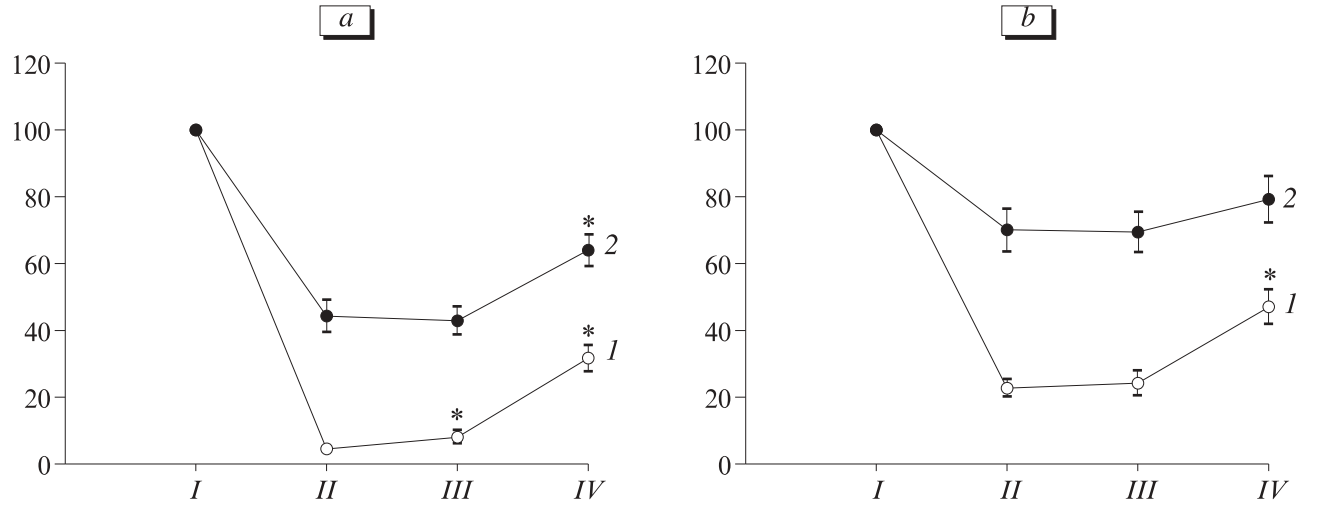

Fig. 1. Time course of nuclear (*a*) and cytoplasmic (*b*) androgen receptor concentrations in the preoptic/anterior hypothalamic area in gonadectomized (*1*) and hemigonadectomized (*2*) male rats after 10-day injections of dehydroepiandrosterone (DHEA). Ordinate: receptor level (%) in comparison with intact animals (100%). *I*) intact group; *II*) control; *III*) 0.1 mg/kg DHEA; *IV*) 0.7 mg/kg DHEA. *$p<0.05$ compared to the control.

After injection of labeled Δ^4-androstendione and DHEA radioactive testosterone appears in the serum and urine [7]. The liver is believed to be the place of these transformations. The fact that single injection of a low dose (0.1 mg/kg) of DHEA caused no appreciable changes in the nuclear AR fraction responsible for biological effect of the hormone also indicates the involvement of this mechanism. It seems that 0.1-0.7 mg/kg hormone is insufficient for the formation of necessary level of testosterone, which, in turn, can initiate the synthesis of receptors. Repeated injections for 10 days lead to accumulation of testosterone sufficient for initiation of the nuclear and cytoplasmic AR.

The effect of DHEA on the hypothalamus can also be due to its genome effect, leading to AR synthesis in the POAH cells, and to the non-genome modulatory effect of DHEA on the level of neuromediators in the hypothalamus and other brain structures functionally related to the hormone-producing hypothalamic nuclei. It was shown that non-genomic mechanism of the neurosteroid effect, including interaction with the cell membrane receptors and activation of second messengers, participates in allopregnenolone suppression of gonadotropin releasing hormone-dependent release of follicle stimulating hormone the culture of pituitary cell [2]. The neurosteroid genomic receptor participates in the mechanism of another neurosteroid (progesterone) effect on the secretion of gonadotropin releasing hormone [12]. Inhibitors of protein transcription and synthesis, for example, actinomycin D and cycloheximide, completely block delayed genomic effects of neuroactive steroids [6].

Our experiments show that the genomic and non-genomic modulatory effects of DHEA in the studied doses are presumably not involved in the effect of this neurosteroid on the level of AR in the hypothalamus of gonadectomized rats. Otherwise, low doses (0.1 mg/kg) of the hormone or its single injection in doses of 0.1-07 would have modulated the level of AR similarly as after 10-day treatment with the neurosteroid in a dose of 0.7 mg/kg.

The increase in the number of intracellular AR in the hypothalamus is important for functioning of the hypothalamo-pituitary-gonadal system feedback mechanism under conditions of testosterone deficiency and for the recovery of adequate sexual behavior [9]. Injection of DHEA has other biological effects, including improvement of memory, relief of anxiety and depression, and optimization of cognitive function [11].

Hence, the increase in the number of nuclear and cytoplasmic receptors of sex hormones in the hypothalamic nuclei under the effect of DHEA partially levels testosterone deficiency in the blood after gonadectomy. The substitute effect of low-dose DHEA recommends it for practical medicine, as it is free from side effects of steroid hormones usually associated with testosterone in therapeutic doses. The use of DHEA together with testosterone in endocrine diseases involving androgen deficiency will permit using lower doses of testosterone and thus reduce the side effect of this hormone. DHEA can be used for potentiation of the therapeutic effects of sex hormone preparations in order to restore the reproductive function and adequate sexual behavior in conditions associated with androgen deficiency.

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