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Dehydroepiandrosterone Regulates Insulin-like Growth Factor-1 System in Adult Rat Hypothalamus

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Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S) exert multiple effects in rodent and human brain. Several findings suggest that insulin-like growth factor-1 (IGF-1) is involved in the actions of DHEA. In this study, we assessed whether systemic administration of DHEA regulates the IGF-1 system in the hypothalamus, hippocampus, cerebral cortex, and cerebellum of adult rats. DHEA resulted in a significant reduction in IGF-1 receptor protein levels. This effect was dose dependent and restricted to the hypothalamus. In contrast to IGF-1 receptor, IGF-1-binding protein 2 levels were unaffected by DHEA treatment. IGF-1 levels were significantly increased in the hypothalamus of the rats treated with DHEA, whereas IGF-1 serum levels were not affected by DHEA. The effects of DHEA on the hypothalamic IGF-1 system may be highly relevant to the control and maintenance of hypothalamic neuroendocrine function.

Key Words: Dehydroepiandrosterone; insulin-like growth factor-1; IGF-1 receptor; IGF-binding protein-2; hypothalamus.

Introduction

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S) exert multiple effects in rodent and human brain *(1,2)*. These effects may be mediated in part by a direct action of DHEA on neurons and glial cells as well as by its conversion to androgens or estrogens *(1–3)*. DHEA and DHEA-S modulate the function of neurotransmitter membrane receptors, such as NMDA, $GABA_A$, and sigma receptors *(1,2)*. Furthermore, DHEA and DHEA-S can interact with the synthesis and/or release of neurotransmitters such as norepinephrine, epinephrine, dopamine, and β-endorphin in the hypothalamus *(4)*. DHEA has anxiolytic effects, reduces aggressive behavior in mice, enhances memory, and may decrease food intake by regulating serotonin and leptin levels *(1,2,4,5)*. DHEA treatment reduces fat accumulation, induces glucose uptake in adipocytes, and protects against insulin resistance in male rats *(6,7)*. Furthermore, DHEA regulates the response of central nervous tissue to injury *(8)* and protects neurons against degeneration induced by glucocorticoids, glutamate, oxidative stress, anoxia, ischemia, and hyperglycemia *(9–14)*.

DHEA levels decline with aging *(15)*, and DHEA replacement therapy in aged men and women may increase muscle strength and lean body mass, activate immune function, improve libido, and enhance quality of life *(16,17)*. DHEA is free of the potential risk of breast and uterine cancer, while it stimulates bone formation and vaginal maturation in postmenopausal women *(18)*. Furthermore, DHEA treatment can exert a beneficial influence by reversing the decrease in hypothalamic corticotropin-releasing hormone and gonadotropin-releasing hormone and pituitary proopiomelanocortin mRNA expression that occurs as a consequence of aging *(19–21)*.

Several findings suggest that insulin-like growth factor-1 (IGF-1) is involved in the actions of DHEA. IGF-1 is produced in the liver and other organs in response to growth hormone *(22)* and exerts hormonal, paracrine, and autocrine effects in different tissues, including the brain *(23)*. In the central nervous system (CNS), IGF-1 acts as a trophic factor during development *(24)* and as a neuromodulator in adult life *(25)*. IGF-1 receptors (IGF-1Rs) are widely expressed in the adult brain where IGF-1 regulates a variety of functional events, including neurogenesis, synaptic plasticity, learning and memory, and the mechanisms of response to injury and neurodegeneration *(23–27)*. IGF-1 increases the production of DHEA by adrenal cortical cells *(28)*. Furthermore, DHEA induces IGF-1 synthesis by granulosa cells *(29)*, and IGF-1 levels have been reported to be enhanced by the administration of DHEA in humans *(30)* and rats *(31)*. In addition, both DHEA and IGF-1 increase in plasma after exercise and decrease with aging *(32,33)*. In spite of these potential links between DHEA and IGF-1 in the periphery, it is unknown whether this steroid may affect the IGF-1 system in the brain. Therefore, in the present study we assessed whether systemic administration of DHEA regulates brain levels of IGF-1, the IGF-1R, and the IGF-binding protein-2 (IGFBP-2), the most abundant IGFBP in the adult CNS *(34,35)*.

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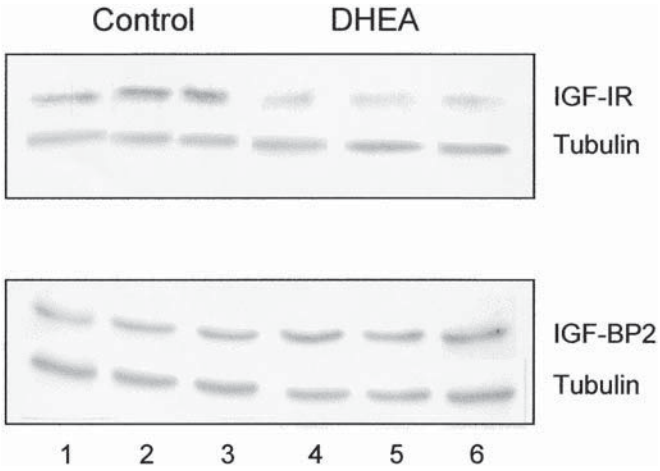

Fig. 1. Representative Western blots of IGF-1R and IGF-BP2 in adult male rat hypothalamus. Lanes 1–3, control animals treated with vehicle; lanes 4–6, animals treated with DHEA (50 mg/kg body wt). Tubulin was used as a loading control.

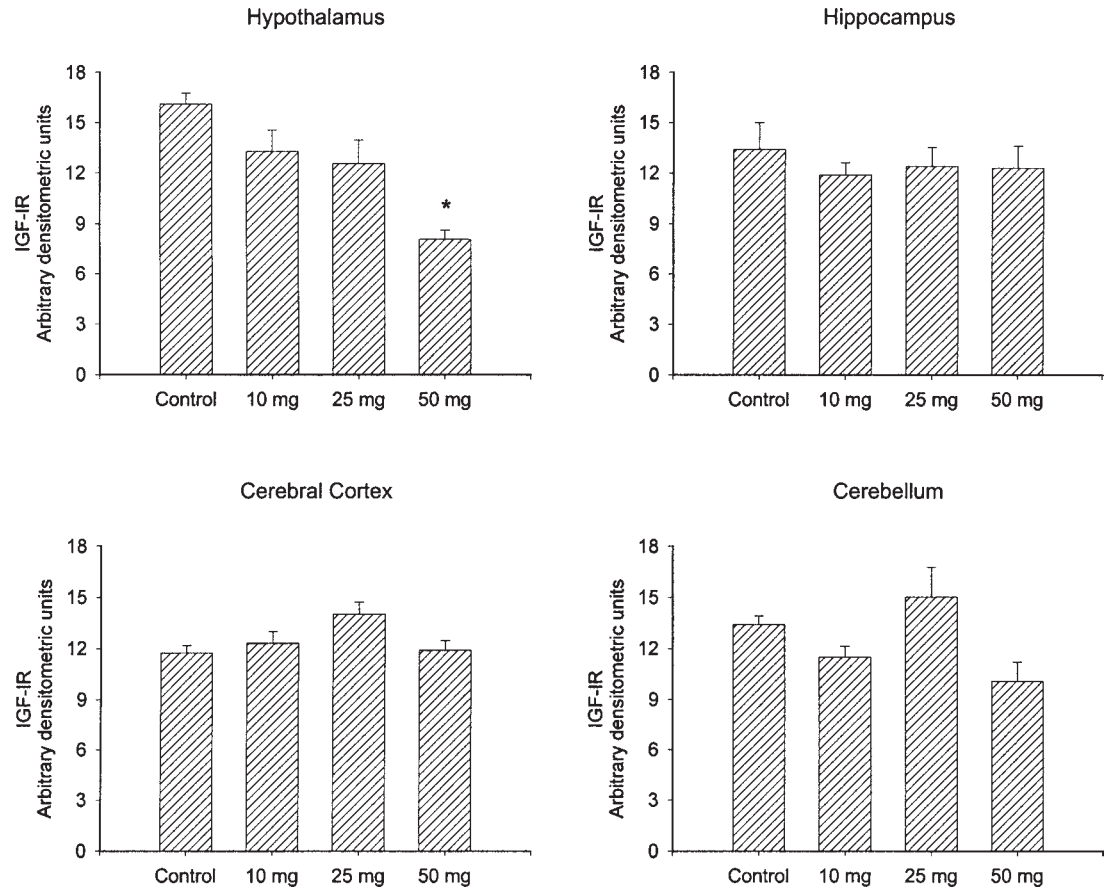

Fig. 2. Effect of DHEA treatment (10, 25, or 50 mg/kg body wt) on IGF-1R protein levels in hypothalamus, hippocampus, cerebral cortex, and cerebellum. Data are expressed as arbitrary densitometric units. *Significant difference vs control value ($p < 0.005$).

Results

DHEA resulted in a significant reduction in IGF-IR protein levels. This effect was region and dose dependent since it was observed exclusively in the hypothalamus ($p < 0.005$) and only with the highest tested dose (50 mg/kg) of DHEA (Figs. 1 and 2). In contrast to IGF-IR, IGF-BP2 protein levels were unaffected by DHEA treatment (Figs. 1 and 3).

Considering that the DHEA-induced downregulation of hypothalamic IGF-IR may be related to changes in local IGF-1 levels, a group of male adult rats was studied to

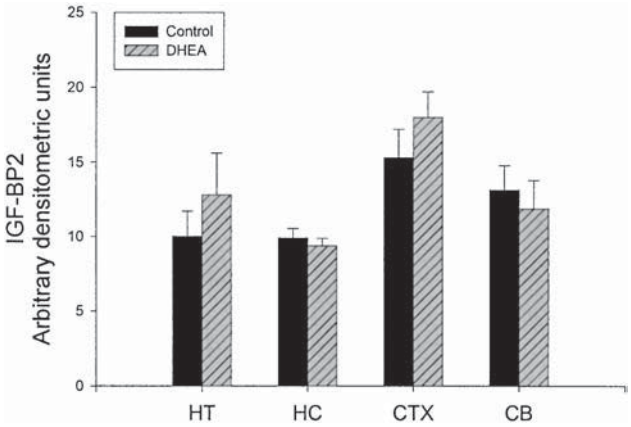

Fig. 3. IGF-BP2 protein levels in hypothalamus (HT), hippoccampus (HC), cerebral cortex (CTX), and cerebellum (CB) after DHEA treatment (50 mg/kg body wt). Data are expressed as arbitrary densitometric units. DHEA treatment did not affect IGF-BP2 expression. Lower doses of DHEA (10 and 25 mg/kg body wt) were also without effect (not shown).

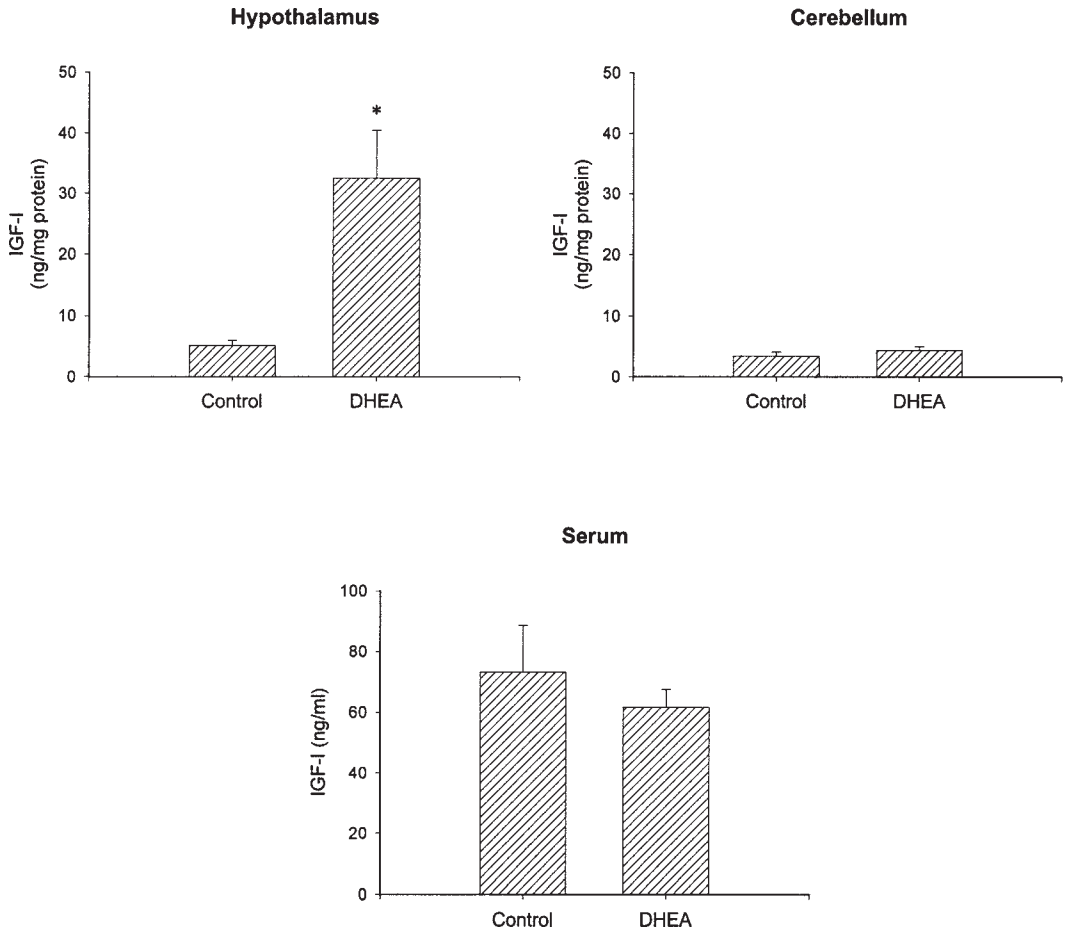

Fig. 4. IGF-1 levels in hypothalamus, cerebellum, and serum in response to DHEA (50 mg/kg). *Significant difference vs control value ($p < 0.005$).

ascertain the possible effect of DHEA on hypothalamic and systemic IGF-1 levels. A dose of DHEA of 50 mg/kg was selected, since this is the dose that was able to change IGF-1R protein levels in the hypothalamus. The cerebellum was used as a tissue control that did not show differences in IGF-1R after DHEA treatment. IGF-1 serum levels were not affected by DHEA treatment (Fig. 4). By contrast, IGF-1 levels were significantly increased in the hypothalamus of the rats treated with DHEA, while IGF-1 levels in the cerebellum were unaffected (Fig. 4).

Discussion

Our findings indicate that the systemic administration of DHEA to male rats results in the downregulation of IGF-1R expression in the hypothalamus. The effect seems to be specific for this brain region, since IGF-1R was not affected in other brain areas studied: the hippocampus, cerebral cortex, and cerebellum. Furthermore, DHEA treatment resulted in the upregulation of IGF-1 levels. This effect appears also to be restricted to the hypothalamus, since IGF-1 levels in the cerebellum and in plasma were not affected by DHEA treatment. To our knowledge, this is the first demonstration of modulation of the IGF-1 system by DHEA in the CNS.

The effect of DHEA on IGF-1Rs and IGF-1 levels was obtained using a dose of the steroid that is within the range used in previous studies *(36–38)*. The dose and pattern of administration of DHEA used in our study have been previously shown to alter brain function in rats, affecting behavior *(38)* and food intake *(39)*. DHEA may exert its actions on the CNS by a variety of mechanisms, including direct effects on neurotransmitter receptors as well as by its conversion to other steroids. Hypothalamic astrocytes in culture metabolize DHEA to testosterone and estradiol *(3)*. Furthermore, DHEA is mainly converted to 7α- and 7β-hydroxylated metabolites in primary cultures of rat hippocampus *(40)*. Different mechanisms of action or different rates of local DHEA metabolism may explain the regional differences observed in the action of the steroid on the IGF-1 system. The specific decrease in IGF-1R in the hypothalamus after DHEA treatment may be the result of a downregulation induced by the increased levels of IGF-1 in this brain area. In turn, the specific increase in IGF-1 levels in the hypothalamus may be the result of a local induction of IGF-1 synthesis. Moreover, as mentioned, DHEA may be converted to androgens or estrogens, and these steroids regulate the accumulation of IGF-1 in the hypothalamus *(41,42)*.

The effect of DHEA on the hypothalamic IGF-1 system may have important implications for neuroendocrine control. IGF-1Rs are expressed in many neuronal populations in the preoptic area and the hypothalamus, including the arcuate, the supraoptic, and the paraventricular nuclei *(43)*. IGF-1 levels increase during puberty in the hypothalamus of male and female rats and fluctuate in the hypothalamus of adult females in concert with the different stages of the estrous cycle *(44)*. In the preoptic area, IGF-1 may affect neuroendocrine events by the regulation of α(1)-adrenergic receptor signaling *(45)*. IGF-1 also affects axonal growth in hypothalamic magnocellular neurons *(46)* and synaptic plasticity in the hypothalamic arcuate nucleus *(47)*. Furthermore, IGF-1, acting on the hypothalamus, may regulate gonadotropin secretion *(48,49)*. Therefore, the effects of DHEA on the hypothalamic IGF-1 system may be highly relevant to the control and maintenance of hypothalamic neuroendocrine function.

Materials and Methods

Animals and Treatments

Wistar albino male rats (Harlan Interfauna Iberica, S.A., Barcelona, Spain) were kept in a 12:12-h light/dark cycle in a temperature-controlled environment (25ºC) and received food and water ad libitum. Animals were handled following the European Union (86/609/EEC) guidelines, and special care was taken to minimize the number of animals used and their suffering. Adult animals (250–300 g) were injected intraperitoneally with 10, 25, or 50 mg/kg of DHEA (dehydroisoandrosterone, 5-androster-3β-ol-one; Sigma, St. Louis, MO) or with vehicle (20% β-cyclodextrin; Sigma). Animals were killed by decapitation 24 h after the injections and their brains were quickly removed. The hypothalamus, hippocampus, cerebral cortex, and cerebellum were dissected out and frozen on dry ice. For radioimmunoassay (RIA) determinations, the trunk blood was collected and centrifuged, and the serum was stored at –20ºC until assayed for IGF-1 concentrations.

Western Blotting

The tissues were homogenized with lysis buffer, pH 7.4, containing 20 m*M* Tris HCl, 150 m*M* NaCl, 1 m*M* EDTA, 10% glycerol, 1% Nonidet P40 (Boehringer Mannheim), 200 μ*M* phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 25 μg/mL leupeptin, and 100 μ*M* vanadate (Sigma). Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (8–12%) was carried out using a miniprotein system (Bio-Rad, Hercules, CA) with broad-range molecular weight standards (Bio-Rad). Protein (100 μg) was loaded in each lane with loading buffer containing 0.375 *M* Tris (pH 6.8), 50% glycerol, 10% SDS, 0.5 *M* dithiothreitol, and 0.002% bromophenol blue. Samples were heated at 100ºC for 3 min prior to gel loading. After electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher & Schuell) using an electrophoretic transfer system (mini Trans-blot Electrophoretic Transfer Cell) at 110 V for 1–2 h. The membranes were then washed with TTBS (20 m*M* Tris-HCl, 7.5 pH; 150 m*M* NaCl; 0.05% Tween-20, pH 7.4) and 8% nonfat dry milk for 90 min. The membranes were incubated overnight at 4ºC with the primary antibodies diluted in TTBS. A rabbit polyclonal antibody for IGF-1 receptor (1:1000) (C20; Santa Cruz Biotechnology, Santa Cruz, CA) and a rabbit polyclonal antibody for IGF-BP2 (1:1000) (Upstate Biotechnology) were used. After washing, the membranes were incubated for 2 h at room temperature with secondary antibody (1:20,000) (anti-rabbit IgG peroxidase conjugated; Jackson ImmunoResearch), washed with TBS (20 m*M* Tris-HCl; 150 m*M* NaCl, pH 7.5), and developed with the chemiluminescence ECL Western blotting system (Amersham) followed by apposition of the membranes to autoradiographic films (Hyperfilm ECL; Amersham). Autoradiographic films were analyzed using the Molecular

Dynamics Image Quant software version 3.22 (computing densitometer model 300A). The membranes were incubated with stripping buffer (62.5 m*M* Tris-HCl, 2% SDS, 100 m*M* 2-mercaptoethanol) for 30 min at 70ºC and, using the same procedures as just described, were incubated with a mouse polyclonal antibody for β-III tubulin (diluted 1:1000; Promega, Madison, WI) and the corresponding secondary antibody (anti-mouse IgG peroxidase conjugated, diluted 1:20,000; Jackson ImmunoResearch). The results from each membrane were normalized to the β-III tubulin values. To minimize interassay variations, samples from all experimental groups were processed in parallel. An example of the bands obtained is shown in Fig. 1.

IGF-1 Radioimmunoassay

The RIA procedure has been described in detail previously *(50)*. IGF-1 was radioiodinated by the lactoperoxidase method. Assay sensitivity was 0.8 ng of IGF-1/mL and the intra- and interassay coefficients of variation were 13.5 and 1.7%, respectively. The anti-IGF-1 polyclonal antibody used shows very high binding affinity for IGF-1 *(51)*. Serum samples were extracted using Millipore C18 Sep-Pak cartridges to remove the endogenous IGF-BPs and dried in a vacuum centrifuge (Savant, Farmingdale, NY). The recovery of the Sep-Pak purification procedure was 75%. Tissue samples were homogenized with 1 *N* acetic acid, boiled for 20 min, and lyophilized. Samples were reconstituted in RIA buffer (0.05 *M* phosphate with 0.4% bovine serum albumin, pH 7.6) and assayed.

Statistical Analyses

Data are expressed as the mean ± SEM. A one-way analysis of variance was used for multiple statistical comparisons. The Tukey test was used to determine significant differences between two groups. Four to six rats were used for each experimental group. Differences with a value of $p < 0.05$ were considered significant.

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