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The protective effect of frontal cortex dehydroepiandrosterone in anxiety and depressive models in mice

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Abstract

We aimed to verify whether DHEA, a neuoroactive neurosteroid, has a protective role in preventing the occurrence or enhancement of the severity of depression and anxiety in mice. Four groups were tested: controls, mice possessing significantly high frontal cortex DHEA levels, achieved by repeated DHEA injections (1.6 mg/Kg, i.p.), mice that have significantly low frontal cortex DHEA levels, consequent to castration and mice possessing significantly low frontal cortex DHEA levels, treated with DHEA to reverse its level to normal, achieved by castration and repeated DHEA injections (0.4 mg/Kg, i.p.). The Forced Swim Test to determine depressive-like and the Elevated Plus Maze (EPM) to evaluate anxiety-like behaviors, were used. We found that DHEA had an anti-depressive-like effect, as shown by a decreased immobility time in mice possessing a high level of frontal cortex DHEA and increased immobility time in mice that have a low frontal cortex DHEA level. DHEA also demonstrated an anti-anxiety-like effect, as shown by the open-arm time in EPM, which correlated with DHEA level. Mice with significantly low DHEA levels when restored to normal, did not differ from controls. In conclusion, high levels of DHEA have an anti-anxiety-like and an anti-depressive-like effect in mice and those with low levels of frontal cortex DHEA are more vulnerable to depression and/or anxiety.

Keywords: Dehydroepiandrosterone (DHEA); Neurosteroids; Depression; Anxiety; Mice; Forced Swim Test (FST); Elevated Plus Maze (EPM); Behavioral disorders

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) together defined as DHEA/S are the major secretory products of human and fetal adrenal glands (Friess et al., 2000). DHEA and in parallel, DHEAS, are also produced in the brain, thus termed neurosteroids (Stoffel-Wagner, 2001; Zwain and Yen, 1999; Brown et al., 2000). In contrast to peripheral steroids, which function through binding to intracellular receptors and act as transcriptional factors in the regulation of gene expression, neuroactive steroids modulate ligand-gated ion channels via non-genomic mechanisms through the cell surface receptors (Rupprecht et al., 2001) and rapidly alter neuronal excitability (requiring milliseconds to minutes). The term 'neuroactive' describes steroids that possess this neuronal modulatory activity. Modulation of neurotransmitter receptors by neuroactive steroids includes γ-aminobutyric acid type A ($GABA_A$), serotonin ($5\text{-}HT_3$), nicotinic acetylcholine, glycine, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), sigma 1 and kainate receptors (Paul and Purdy, 1992).

Neurosteroids are known to be involved in conditioned behavioral processes which are regulated by psychological processes, such as response to stressful events, cognition, anxiety, aggression, depression, and in other regulatory behaviors, such as sleep, ingestion and reinforcement (Engel and Grant, 2001). DHEA appears to have a wide range of beneficial effects when administered to humans and rodents. Several examples include anticarcinogenic, antidiabetic and immunomodulatory activities.

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In addition, DHEA improves general well being, inhibit atherosclerosis, promote neurogenesis, improve memory and cognition and exhibit anti-depressive, anxiolytic, antiagressive and antipsychotic properties (Binello and Gordon, 2003; Wolf and Kirschbaum, 1999; Kimonides et al., 1998). The total positive physiological and affective influences that are associated with DHEA support the assumption that DHEA has a significant role in behavior regulation.

From our group's previous studies it appears that high levels of DHEA and DHEAS have a protective role and attenuate the severity of behavioral disorders such as depression, hyperactivity and psychosis, and may function as part of the therapeutic mechanism of several medications. In teenagers with Attention Deficit Hyperactivity Disorder (ADHD) we found an inverse correlation between blood DHEA levels and disorder severity (Strous et al., 2001). In depressed patients, elevated basal DHEAS circulatory levels was associated with resistance to electroconvulsive therapy (Maayan et al., 2000). Combat-related posttraumatic stress disorder (PTSD) was accompanied by an elevated level of DHEA/S (Spivak et al., 2000). Chronic methylphenidate treatment of ADHD children increases the level of both DHEA and DHEAS, possibly as part of its therapeutic effect (Maayan et al., 2003). In addition, DHEA treatment attenuates the negative symptoms in antipsychotic-treated schizophrenia patients (Strous et al., 2003). Other studies support the possibility of DHEA behavioral neuroprotective activity (Veiga et al., 2003; D'Astous et al., 2003; Lapchak et al., 2000; Lapchak and Araujo, 2001).

DHEA is a precursor of estradiol (E2). Thus, DHEA treatment may lead to an increase in E2 levels. As estrogens are known for their effect on brain development and neuroprotection (Rao and Kolsch, 2003; Tam et al., 2002; Azcoitia et al., 2001; Wolf et al., 1999; Cyr et al., 2000; Veiga et al., 2003; Estrada-Camarena et al., 2003), in studies examining the protective effect of DHEA activity, elevation in the E2 level must be prevented.

The aim of this study is to explore the possible beneficial effect of DHEA in mice models of depression [using the Forced Swim Test (FST)] and anxiety [using the Elevated Plus Maze (EPM)]. Alleviation of depression and anxiety after DHEA elevation, despite the elimination of the possible effects of increased E2, would suggest that high DHEA has a psychotropic activity in these behavioral disorders.

2. Methods

2.1. Animals

Male, ICR, sexually mature mice (7–8 weeks old) weighing 30–35 g, were habituated to the housing conditions for two days. Mice were housed 5 per cage on a reversed 12-h light: 12-h dark cycle. Food and water were available ad libitum and the temperature was maintained at 22°±1 °C. The study was approved by the Tel Aviv University Committee for Experiments in Animals and the experiments were conducted according to the guidelines of the Tel-Aviv University Committee for care and treatment of animals.

All mice were anesthetized with diethyl ether before any surgical procedure. Mice tested for their steroid levels, were decapitated at the end of the experiment, whole blood was collected and serum was separated. Brains were removed and frontal cortex (FC) was dissected and assessed. All samples were kept in −20 °C till assayed. Mice were divided into two main groups: one for biochemical assays and one for behavioral tests.

Each group was divided into 4 subgroups after two days of habituation, as follows:

a. Sham-castrated mice, serving as controls (ctl)
b. Castrated mice (ctx) — serving as mice having significantly decreased FC-DHEA level.
c. Castrated mice treated with DHEA (ctx-DHEA) — castration removes not only DHEA but also other steroids and non-steroidal components. The aim of testing the behavior of castrated mice, to which only DHEA level is restored, is to validate the assumption that it is the deficiency of DHEA, not the lack of other testicular components removed as a result of castration, that caused behavioral changes after this procedure.
d. Sham-castrated mice treated with DHEA (DHEA) — serving as mice having significantly increased FC-DHEA level.

2.2. Treatment and procedures

2.2.1. DHEA treatment

DHEA was dissolved in 2–3 drops of DMSO and diluted with saline to yield 0.4 mg DHEA/Kg body weight (ctx+DHEA) injected in a volume of 200 µl. This dose is needed to achieve a physiological concentration of FC-DHEA in castrated mice. The DHEA group was injected with 1.6 mg DHEA/Kg body weight (DHEA) injected in a volume of 200 µl, a dose needed to achieve a significant increase in frontal cortex DHEA. Ctl and ctx mice were injected with 200 µl saline. All mice were injected daily, i.p., for 5 consecutive days at 8.a.m.

2.2.2. Steroid extraction from frontal cortex

Rats were decapitated between 9:00 to 11:00 a.m. brains were removed, rinsed of blood and carefully dissected to remove the frontal cortex (FC) which is important in behavioral regulation. FC was homogenized (Politron, PCU, Lucerne, Switzerland) in 4 ml absolute ethanol and placed in −20 °C for 24 h for deproteinization and free steroid extraction. Samples were then centrifuged at 10,000 g for 30 min at 4 °C. The organic phase, which contained both DHEA and E2, was removed and evaporated till dryness. Samples were dissolved in either 120 µl standard 0 (supplied by the DHEA kit) from which 100 µl were taken for estimating the level of DHEA or 220 µl standard 0 (supplied by the E2 kit), 200 µl of which were removed for estimating the level of E2.

Recovery of ethanol extraction was determined using labeled radioisotopes and was found to be 88%–91%; homogenization in buffer and extraction of both E2 and DHEA using diethyl ether resulted in a lower recovery rate. Ethanol-extracted steroids were evaporated to dryness and dissolved in buffer steroids or 0 ng/ml standard of the RIA kit containing the appropriate milieu needed for the assay, but with 0 ng/ml of the tested steroids.

In addition to testing the recovery of ethanol extraction, recovery and linearity of dilution were tested. Recovery was tested by adding known quantities of steroids to the frontal cortex extract; the recovery was found to be 84–88%. Linearity of dilution was tested using 0 ng/ml steroid standard and the observed values matched the expected ones by more than 88%. The volume of frontal cortex extracts taken for evaluation was determined to reach a final concentration suitable for the sensitivity and range of the RIA kit.

2.2.3. Steroid extraction from serum

0.5 ml whole blood samples, collected immediately after decapitation, were centrifuged at 1000 g for 10 min. Free steroids were extracted by 5.0 ml diethyl ether, centrifuged at 1000 g for 3 min and placed at −70 °C for 1 h in order to freeze the aqueous solution. The organic phase was removed, evaporated till dryness and then dissolved in 120 µl standard 0 (supplied by the DHEA kit) 100 µl of which were removed to estimate the level of DHEA or in 220 µl standard 0 (supplied by the E2 kit) from which 200 µl were taken for estimating the level of E2.

2.2.4. Castration

Two days after habituation, mice were anesthetized with ether and both testes were cut through an incision in the scrotum and removed. Sham operated mice had an incision in the scrotum which was immediately closed.

2.3. Steroid determination

2.3.1. DHEA

DHEA was determined using DHEA-DSL 9000Active TM DHEA coated tube radioimmunoassay (RIA) kit (Diagnostic System Laboratories, Webster, Texas, USA). The detection limit of the assay is 0.02 ng/ml; assay variability is 10.2% between runs and 5.6–10.6% within runs according to the level of DHEA in the sample; cross reactivity with other steroids is <0.2%. All tests were conducted simultaneously in order to avoid inter assay differences.

2.3.2. E2

E2 was determined using the E2 Ultra-Sensitive Estradiol DSL-4800 radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, Texas, USA). The detection limit of the assay is 2.2 pg/ml; assay variability is 7.5–12% between runs and 6.5–9.0% within runs according to the level of E2 in the sample; cross reactivity with other steroids is <2.5%. All tests were conducted simultaneously in order to avoid inter assay differences.

2.4. Behavioral tests

All behavioral tests were performed on the 5th day, as we found in a previous study that this was the earliest day on which both serum and frontal cortex DHEA levels in castrated mice are significantly lower than in the control group (Maayan et al., 2005).

2.4.1. Forced Swim Test (FST)

Mice were placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 22–23 °C. The mice were forced to swim for 5 min. and the duration of immobility time during the last 4 min was measured. The mouse was considered as immobile when it stopped struggling and moved only to remain floating, keeping its head above the water. DHEA was administered 30 min before the session. The apparatus was rinsed with water after each test.

2.4.2. Elevated Plus Maze (EPM)

The plus maze consists of two open-arms (30×5 cm, surrounded by a 0.5 cm-high border) and two closed arms (30×5 cm, surrounded by a 10 cm-high walls), with the two pairs of identical arms, which emerged from a central platform (5×5 cm), positioned opposite each other. The base of the arms and central platform were colored in grey, while the walls of the closed arms were colored in black. The apparatus was elevated 50 cm above the floor. The mice were routinely tested during the first half of the light phase of their dark/light cycle. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open-arms, and letting it move freely. Each session lasted 5 min starting 30 min after the last DHEA administration. Mouse behavior was videotaped by a videocamera placed above the apparatus. The maze was cleaned with alcohol and rinsed with water after each test.

2.5. Statistical analysis

Data was analyzed using analysis of variance (ANOVA) and Student's *t* test, as appropriate. The criteria for statistical significance were $p<0.05$. Results are expressed as mean±SEM.

3. Results

3.1. The influence of castration and DHEA treatment on DHEA level

DHEA was injected in two doses: 1.6 mg/Kg body weight (DHEA group) in order to reach a significantly higher level of frontal cortex DHEA compared to control mice and a dose of 0.4 mg/Kg body weight in order to restore the level of brain DHEA in castrated mice (ctx+DHEA) (see Methods). The ctx+DHEA group serves as a second control in which the mice were castrated but have no deficit in DHEA level.

3.1.1. A. Serum

Serum DHEA level in the high dose DHEA-treated group (1.6 mg/Kg body weight) was significantly higher than in ctl and ctx+DHEA groups (both $p<0.05$) and ctx group ($p<0.001$).[$F(3,35)=6.177$; $p=0.0018$] (Fig. 1A). Each group consisted of 9–10 mice.

3.1.2. B. Frontal cortex (FC)

DHEA level in the FC of the ctx group was significantly lower than ctl ($p<0.05$), ctx+DHEA ($p<0.01$) and DHEA

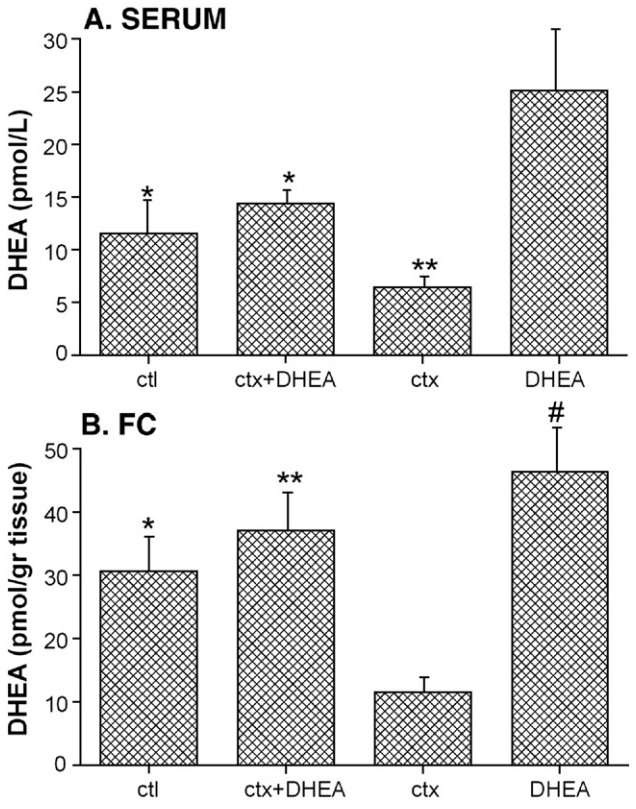

Fig. 1. The influence of castration and DHEA treatment on DHEA level. Serum (n=9–10 in each group). ★p<0.05 vs DHEA. ★★p<0.001 vs DHEA. B. Frontal cortex (FC) (n=8–9 in each group). ★p<0.05 vs ctx. ★★p<0.01 vs ctx. # p<0.001 vs ctx.

groups (p<0.001) [F(3.31)=7.605; p=0.0006] (Fig. 1B). Each group consisted of 8–9 mice.

3.2. The influence of a high dose of DHEA on frontal cortex E2 level

No significant change in brain E2 was observed after DHEA treatment (1.6 mg DHEA/Kg body weight) (Fig. 2) (t=1.075; df=15; p=0.27). Each group consisted of 8–9 mice.

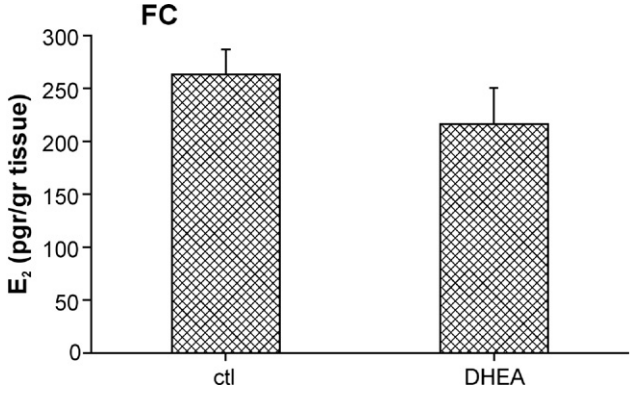

Fig. 2. The influence of DHEA treatment on frontal cortex estradiol (E2) level (n=8–9 in each group).

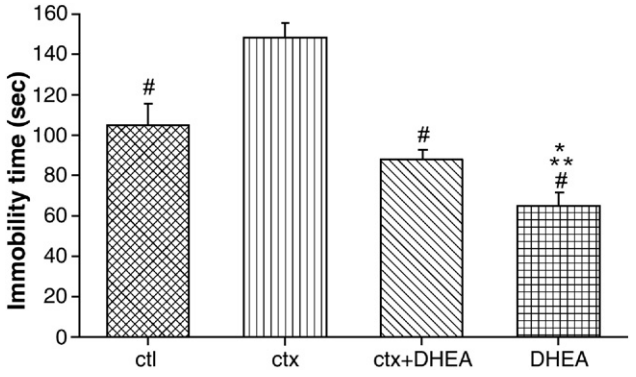

Fig. 3. The influence of DHEA treatment on depression-like behavior as measured by FST. (n=16–18 in each group). ★p<0.05 vs ctx+DHEA. ★★p<0.01 vs ctl. # p<0.001 vs ctx.

3.3. The influence of DHEA treatment on depression-like behavior, as measured by FST.

In DHEA treated mice (DHEA) displaying a significantly higher level of DHEA, immobility time was shorter than ctl (p<0.01), ctx +DHEA (p<0.05) and ctx (p<0.001) groups (Fig. 3). In ctx mice, which had significantly lower levels of frontal cortex DHEA, immobility time was longer than ctl, ctx+DHEA and DHEA groups (p<0.001). No difference in immobility time was observed when the frontal cortex DHEA level was restored in ctx mice (ctx+DHEA) compared to ctl [(ctx+DHEA) vs ctl, p>0.05); ANOVA for all 4 groups: F (3,59)=21.234; p<0.0001] (Fig. 3). Each group consisted of 16–18 mice.

3.4. The influence of DHEA treatment on anxiety-like behavior, as measured by EPM

Open-arm time in EPM is inversely correlated with anxiety-like behavior. As can be seen in Fig. 4, the open-arm time in mice treated with high dose of DHEA (DHEA) was significantly longer than ctl and ctx+DHEA (both p<0.05) and ctx (p<0.001). Ctx mice showed shorter open-arm time (higher anxiety-like behavior), vs both ctl and ctx+DHEA groups (p<0.05). No

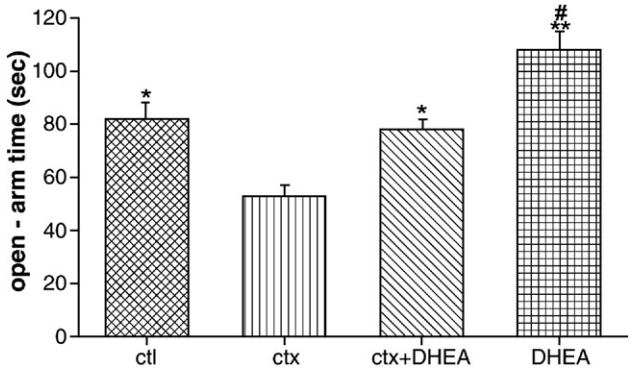

Fig. 4. The influence of DHEA treatment on anxiety-like behavior as measured by EPM. (n=16–18 in each group). ★p<0.05 vs ctx. ★★p<0.001 vs ctx. # p<0.05 vs both ctl and ctx+DHEA.

significant difference was found between ctx+DHEA and ctl groups [$F(3,62)=8.498$; $p<0.0001$]. Each group consisted of 16–18 mice.

4. Discussion

The principal finding of this study was the observation that DHEA may have a protective effect against the development of both depression and anxiety in a mice model. A significant decrease in DHEA levels increased depression- and anxiety-like behaviors which could be attenuated by restoring frontal cortex DHEA to control levels. Most DHEA studies have been conducted on the protective effect of DHEA in neuronal tissue assays but not in a behavioral assay. D'Astous et al. (2003) showed that DHEA prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP)-induced striatal dopamine (DA) depletion in mice. In the substantia nigra, DHEA prevented the decrease of MPTP-induced dopamine transporter and tyrosine hydroxylase mRNA which indicates a neuroprotective effect on DA neurons. DHEAS was shown to be neuroprotective in a model of reversible spinal cord ischemia (Lapchak et al., 2000) and has a protective effect on hilar neurons against kainic acid neurotoxicity (Veiga et al., 2003). In addition it has been shown to promote the survival of newly formed neurons and to prevent corticosterone-induced suppression (Karishma and Herbert, 2002). Furthermore, it protects certain neuronal populations against neurotoxic insults inflicted by the excitatory amino acid glutamate (Kimonides et al., 1998; Mao and Barger, 1998). As such, DHEAS may be useful in treating brain injury and neurodegenerative diseases in which excitotoxicity is believed to be a major contributor to cell death. DHEA has been shown to have oxygen-free radical scavenger properties that may play a role in its neuronal protective activity (Khalil et al., 2000; Aragno et al., 2000; van Rensburg et al., 2000). Some investigators suggest that the neuroprotective activity of DHEA against excitotoxicity-induced neuronal death is mediated by its conversion to testosterone and further on to estradiol by aromatase (Veiga et al., 2003; Cyr et al., 2000; Rao and Kolsch, 2003; Azcoitia et al., 2001). It has also been hypothesized that 7-alpha-hydroxylation of DHEA mediates its neuroprotective activity (Jellinck et al., 2001; Morfin and Starka, 2001).

In addition to the well-established neuroprotective effect of estradiol (E_2), accumulating data have shown a beneficial effect on cognitive function in elderly women including verbal memory and possibly also frontal lobe mediated cognitive functions (Wolf and Kirschbaum, 1999). E_2 modulates the activity of the dopaminergic and the serotonergic systems which are implicated in the pathophysiology of schizophrenia. In addition, negative symptoms scores in schizophrenia seem to be in inverse correlation with the E_2 level (Rao and Kolsch, 2003). Westlund and Parry (2003) suggest that estrogens may enhance the efficacy of antidepressant drugs such as fluoxetine in menopausal women.

Since E_2 has been suggested as a key steroid in the process of neuroprotection, in order to exclude the possibility that any DHEA-associated protective effect may be due to an increase in frontal cortex E_2 level, we measured it after daily treatment of the mice with high doses of DHEA (sufficient to increase frontal cortex DHEA level; "DHEA" group in the present study). We found no significant increase in frontal cortex E_2 level in the DHEA treated mice. This supports our assumption that DHEA, and not its metabolites, mediate the beneficial effect.

Castration causes a drastic decrease in all testes-synthesized products in addition to its suppressive effect on DHEA levels. Orchiectomy-induced decrease in testosterone may be followed by an increase in the levels of luteinizing hormone, follicular stimulating hormone and gonadotropin releasing hormone due to the lack of negative feedback. In order to exclude the possible involvement of other hormones besides DHEA on the behavioral effect of castration, DHEA was injected repeatedly to castrated mice in a dose which restored frontal cortex DHEA to a control level. As shown in Figs. 3 and 4, restoring frontal cortex DHEA level to normal in castrated mice by exogenous DHEA administration attenuated the severity of both depression- and anxiety-like behaviors, supporting our assumption that it is DHEA, and not any other steroid or hormone, that is responsible for the behavioral changes and protective activity in conditions provoking anxiety and depressive behaviors. Our results are supported by Rasmusson et al. (2004) who found that increased capacity of adrenal DHEA release, as measured by adrenocorticotropic hormone (ACTH) and corticotropin releasing factor (CRF) stimulation tests, is associated with decreased avoidance and negative mood symptoms in women with PTSD. Goodyer et al. (2003) also reported that raised morning cortisol/DHEA ratio (due to a tendency to low DHEA) may predict depression in adolescents.

Our findings are supported by Morgan et al. (2004) who found that the ratio or balance between circulation levels of DHEAS and free cortisol may help buffer against centrally mediated negative effects of stress and that low ratios may be associated with vulnerability to stress-induced symptoms of dissociation. This vulnerability is supported by studies which report reduced levels of DHEA/S in individuals with chronic fatigue syndrome (Scott et al., 1999), depression (Goodyer et al., 1996; Wolkowitz et al., 1999; Michael et al., 2000) anxiety (Labbate et al., 1995), anorexia nervosa (Zumoff et al., 1983) and schizophrenia (Oades and Schepker, 1994). We have reported high serum levels in PTSD (Spivak et al., 2000) which we hypothesized to act as a protective mechanism. Furthermore, we have proposed that this modulatory protective effect may have a role in reducing hyperactivity in attention deficit hyperactivity disorder (Strous et al., 2001), and negative symptoms in schizophrenia patients treated with DHEA (Strous et al., 2003). These results also support our assumption that raising DHEA/S levels is part of the therapeutic effect of some drugs, such as methyl phenidate (Maayan et al., 2003).

DHEA is converted at least partly to DHEAS, which has behavioral effects of its own. Unfortunately, in the present study DHEAS was not measured.

In conclusion, DHEA may have a protective effect against the development of depressive-like and anxiety-like behavior in mice. These results may be relevant to human anxiety and depressive disorders.

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References

Aragno M, Parola S, Brignardello E, Mauro A, Tamagno E, Manti R, et al. Dehydroepiandrosterone prevents oxidative injury induced by transient ischemia/reperfusion in the brain of diabetic rats. Diabetes 2000;49:1924–31.

Azcoitia I, Sierra A, Veiga S, Honda S-I, Harada N, Garcia-Segura LM. Brain aromatase is neuroprotective. J Neurobiol 2001;47:318–29.

Binello E, Gordon CM. Clinical uses and misuses of dehydroepiandrosterone. Curr Opin Pharmacol 2003;3:635–41.

Brown RC, Cascio C, Papadopoulos V. Pathways of neurosteroid biosynthesis in cell lines from human brain: regulation of dehydroepiandrosterone formation by oxidative stress and beta-amyloid peptide. J Neurochem 2000;74:847–59.

Cyr D, Calon F, Morissette M, Grandbois M, Di-Paolo T, Callier S. Drugs with estrogen-like potency and brain activity: potential therapeutic application for the CNS. Curr Pharm Des 2000;6:1287–312.

D'Astous M, Morissette M, Tanguay B, Callier S, Di-Paolo T. dehydroepiandrosterone (DHEA) such as 17beta-estradiol prevents MPTP-induced dopamine depletion in mice. Synapse 2003;47:10–4.

Engel SR, Grant KA. Neurosteroids and behavior. Int Rev Neurobiol 2001;46:321–48.

Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcara C. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. Neuropsychopharmacology 2003;28:83-838.

Friess E, Schiffelholz T, Steckler T, Steiger A. Dehydroepiandrosterone—a neurosteroid. Eur J Clin Invest 2000;30(Suppl 3):46–50.

Goodyer IM, Herbert J, Altham PM, Pearson J, Secher SM, Shiers HM. Adrenal secretion during major depression in 8- to 16-year-olds, I. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. Psychol Med 1996;26:245–56.

Goodyer IM, Herbert J, Tamplin A. Psychoendocrine antecedents of persistent first-episode major depression in adolescents: a community-based longitudinal enquiry. Psychol Med 2003;33:601–10.

Jellinck PH, Lee SJ, McEwen BS. Metabolism of dehydroepiandrosterone by rat hippocampal cells in culture: possible role of aromatization and 7-hydroxylation in neuroprotection. J Steroid Biochem Mol Biol 2001;78:313–7.

Karishma KK, Herbert J. Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and presents corticosterone-induced suppression. Eur J Neurosci 2002;16:445–53.

Khalil A, Fortin JP, LeHoux JG, Fulop T. Age-related decrease of dehydroepiandrosterone concentrations in low density lipoproteins and its role in the susceptibility of low density lipoproteins to lipid peroxidation. J Lipid Res 2000;41:1552–61.

Kimonides VG, Khatibi NH, Svendsen CN, Sofroniew MV, Herbert J. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci U S A 1998;95:1852–7.

Labbate LA, Fava M, Oleshansky M, Zoltec J, Littman A, Harig P. Physical fitness and perceived stress. Relationships with coronary artery disease risk factors. Psychosomatics 1995;36:555–60.

Lapchak PA, Araujo DM. Preclinical development of neurosteroids as neuroprotective agents for the treatment of a neurodegenerative diseases. Int Rev Neurobiol 2001;46:379–97.

Lapchak PA, Chapman DF, Nunez SY, Zivin JA. Dehydroepiandrosterone sulfate is neuroprotective in a reversible spinal cord ischemia model: possible involvement of GABA(A) receptors. Stroke 2000;31:1953–6.

Maayan R, Yagorowski Y, Grupper D, Weiss M, Shtaif B, Kaoud MA, et al. Basal plasma dehydroepiandrosterone sulfate level: a possible predictor for response to electroconvulsive therapy in depressed psychotic inpatients. Biol Psychiatry 2000;48:693–701.

Maayan R, Yoran-Hegesh R, Strous R, Nechmad A, Averbuch E, Weizman A, et al. Three-month treatment course of methylphenidate increases plasma levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) in attention deficit hyperactivity disorder. Neuropsychobiology 2003;48:111–5.

Maayan R, Touati-Werner D, Ram E, Galdor M, Weizman A. Is brain dehydroepiandrosterone synthesis modulated by free radicals in mice? Neurosci Lett 2005;377:130–5.

Mao X, Barger SW. Neuroprotection by dehydroepiandrosterone-sulfate: role of an NFkappaB-like factor. Neuroreport 1998;9:759–63.

Michael A, Jenaway A, Paykel ES, Herbert J. Altered salivary dehydroepiandrosterone levels in major depression in adults. Biol Psychiatry 2000;48:989–95.

Morfin R, Starka L. Neurosteroid 7-hydroxylation products in the brain. Int Rev Neurobiol 2001;46:79–95.

Morgan III CA, Southwick S, Hazlett G, Rasmusson A, Hoyt G, Zimolo Z, et al. Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. Arch Gen Psychiatry 2004;61:819–25.

Oades RD, Schepker R. Serum gonadal steroid hormones in young schizophrenic patients. Psychoneuroendocrinology 1994;19:373–85.

Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.

Rao ML, Kolsch H. Effects of estrogen on brain development and neuroprotection — implications for negative symptoms in schizophrenia. Psychoneuroendocrinology 2003;28:83–96.

Rasmusson AM, Vasek J, Lipschitz DS, Vojvoda D, Mustone ME, Shi Q, et al. An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. Neuropsychopharmacology 2004;29:1546–57.

Rupprecht R, di Michele F, Hermann B, Strohle A, Lancel M, Romeo E, et al. Neuroactive steroids: molecular mechanisms of action and implications for neuropsychopharmacology. Brain Res Rev 2001;37:59–67.

Scott LV, Salahuddin F, Cooney J, Svec F, Dinan TG. Differences in adrenal steroid profile in chronic fatigue syndrome, in depression and in health. J Affect Disord 1999;54:129–37.

Spivak B, Maayan R, Kotler M, Mester R, Gil-Ad I, Shtaif B, et al. Elevated circulatory level of GABA(A) antagonistic neurosteroids in patients with combat-related post-traumatic stress disorder. Psychol Med 2000;30:1227–31.

Stoffel-Wagner B. Neurosteroid metabolism in the human brain. Eur J Endocrinol 2001;145:669–79.

Strous RD, Spivak B, Yoran-Hegesh R, Maayan R, Averbuch E, Kotler M, et al. Analysis of neurosteroid levels in attention deficit hyperactivity disorder. Int J Neuropsychopharmacol 2001;4:259–64.

Strous RD, Maayan R, Lapidus R, Stryjer R, Lustig M, Kotler M, et al. Value of dehydroepiandrosterone (DHEA) augmentation in the management of negative symptoms in schizophrenia. Arch Gen Psychiatry 2003;60:133–41.

Tam J, Danilovich N, Nillson K, Sairam MR, Maysinger D. Chronic estrogen deficiency leads to molecular aberrations related to neurodegenerative changes in follitropin receptor knockout female mice. Neuroscience 2002;114:493–506.

van Rensburg SJ, Daniels WM, van Zyl JM, Taljaard JJ. A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. Metab Brain Dis 2000;15:257–65.

Veiga S, Garcia-Segura LM, Azcoitia I. Neuroprotection by the steroids pregnenolone and dehydroepiandrrosterone is mediated by the enzyme aromatase. J Neurobiol 2003;56:398–406.

Westlund Tam L, Parry BL. Does estrogen enhance the antidepressant effects of fluoxetine? J Affect Disord 2003;77:87–92.

Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. Brain Res Brain Res Rev 1999;30:264–88.

Wolf OT, Kudielka BM, Hellhammer DH, Torber S, McEwen BS, Kirschbaum C. Two weeks of transdermal estradiol treatment in postmenopausal elderly women and its effect on memory and mood: verbal memory changes are associated with the treatment induced estradiol levels. Psychoneuroendocrinology 1999;24:727–41.

Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, et al. Double-blind treatment of major depression with dehydroepiandrosterone. Am J Psychiatry 1999;156:646–9.

Zumoff B, Walsh BT, Katz JL, Levin J, Rosenfeld RS, Kream J, et al. Subnormal plasma dehydroisoandrosterone to cortisol ratio in anorexia nervosa: a second hormonal parameter of ontogenic regression. J Clin Endocrinol Metab 1983;56:668–72.

Zwain IH, Yen SS. Dehydroepiandrosterone: biosynthesis and metabolism in the brain. Endocrinology 1999;140:880–7.