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DHEA Lessens Depressive-Like Behavior via GABA-ergic Modulation of the Mesolimbic System

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Alterations in the levels of dehydroepiandrosterone (DHEA) in the brain can allosterically modulate γ-aminobutyric-acid-type-A ($GABA_AR$), *N*-methyl-D-aspartate (NMDAR), and Sigma-1 (σ1R) receptors. In humans, DHEA has antidepressive effects; however, the mechanism is unknown. We examined whether alterations in DHEA also occur in an animal model of depression, the Flinders-sensitive-line (FSL) rats, with the intention of determining the brain site of DHEA action and its antidepressant mechanism. We discovered that DHEA levels were lower in some brain regions involved with depression of FSL rats compared to Sprague–Dawley (SD) controls. Moreover, DHEA (1 mg/kg IP for 14 days)-treated FSL rats were more mobile in the forced swim test than FSL controls. In the NAc and VTA, significant changes were observed in the levels of the δ-subunit of $GABA_A$, but not of σ1R mRNA, in FSL rats compared to SD rats. The δ-subunit controls the sensitivity of the $GABA_AR$ to the neurosteroid. Indeed, treatment (14 days) of FSL rats with the $GABA_A$ agonist muscimol (0.5 mg/kg), together with DHEA (a negative modulator of $GABA_A$), reversed the effect of DHEA on immobility in the swim test. Perfusion of DHEA sulfate (DHEAS) (3 nM and 30 nM for 14 days) into the VTA and NAc of FSL rats improved their performance in the swim test for at least 3 weeks post-treatment. Our results imply that alterations in DHEA are involved in the pathophysiology of depression and that the antidepressant action of DHEA is mediated via $GABA_ARs$ in the NAc and VTA.
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INTRODUCTION

Depressive disorders are serious illnesses and a public health problem. Major depression, which usually develops early in life and can last a lifetime, impairs the overall occupational and social functioning, thus affecting a patient's quality of life (Angst, 1992). Most antidepressants improve monoaminergic neurotransmission directly or indirectly, and although their pharmacological action is immediate, their therapeutic mechanism is not clear because it takes weeks before symptoms improve and the patient feels better.

Dehydroepiandrosterone (DHEA), a neurosteroid synthesized in the nervous system from cholesterol (Stoffel-Wagner, 2001), has been associated with antidepressive actions. Neurosteroids like other steroids, can regulate transcription via nuclear receptors, but unlike other steroids they can also rapidly influence neurons' excitability by acting on membrane-bound, ligand-gated ion-channels (McEwen, 1991).

Elucidation of the role of neurosteroids in the pathophysiology and treatment of depression has begun recently (van Broekhoven and Verkes, 2003). Depressed patients have lower levels of plasma DHEA sulfate (DHEAS) (Barrett-Connor *et al*, 1999; Goodyer *et al*, 1998) and of saliva DHEA (Fabian *et al*, 2001; Takebayashi *et al*, 1998) than controls. Moreover, lower saliva DHEA levels in the morning correlate negatively with the severity of depression (Michael *et al*, 2000). In elderly remitters, DHEA and DHEAS levels decrease with time, whereas non-remitters and control subjects exhibit normal levels.

Administration of DHEA to depressed patients improves their symptoms (Wolkowitz *et al*, 1997, 1999), with daily treatment improving the overall well-being of the patient by preventing depressive episodes, decreasing fatigue, and increasing libido (Nadjafi-Triebsch *et al*, 2003). Significant improvement also occurs in men and women with midlife-onset major or minor depression upon treatment with DHEA for 6 weeks, according to the Hamilton Depression Rating Scale and the Center for Epidemiologic Studies Depression Scale ratings (Schmidt *et al*, 2005). Early studies showed that DHEA and DHEAS exhibit antidepressant-like

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effects in mice, as determined by the forced swim test (Prasad *et al*, 1997; Reddy *et al*, 1998).

DHEA and its sulfate derivative DHEAS, which is soluble and exhibits a greater potency, are negative allosteric modulators of the γ-aminobutyric-acid receptor ($GABA_AR$; (Demirgoren *et al*, 1991; Majewska *et al*, 1990; McEwen, 1991). However, other reports suggest that DHEAS in some systems may act opposite to DHEA (Park-Chung *et al*, 1999).

DHEA mechanism as antidepressant may act via modulation of the $GABA_ARs$ activity. The GABA-ergic system can mediate depression (Lloyd *et al*, 1989). In depressed patients, cerebral spinal fluid levels of GABA are lower than in healthy controls (Gerner and Hare, 1981; Gold *et al*, 1980). Furthermore, low GABA content is correlated with depression in post-mortem temporal and frontal cortices from patients with Alzheimer's disease (Garcia-Alloza *et al*, 2006). In these patients, a high density of $GABA_ARs$ correlates with the severity of depression. Despite the increased cortical concentration of GABA upon antidepressant treatment of healthy individuals (Bhagwagar *et al*, 2004) and electroconvulsive treatment of depressed patients (Sanacora *et al*, 2003), antidepressants that affect GABA have yet to be developed.

$GABA_ARs$ are heterogeneous pentamers composed of various combinations of α_{1-6},β_{1-4},γ_{1-3},δ,ε,π, and ρ subunits. The subunit composition determines the pharmacological properties of $GABA_AR$. Drugs, including neurosteroids, which interact with the receptors, can affect the subunit mRNA expression (Sun *et al*, 2004). $GABA_AR$ usually contains two α-subunits, two β-subunits, and either a γ or δ-subunit, with the δ-subunit being rare (Sun *et al*, 2004). $GABA_ARs$ containing a δ-subunit, rather than a γ-subunit, are more sensitive to neurosteroids (Belelli *et al*, 2002), and mice lacking $GABA_A$ δ-subunits are less sensitive to neurosteroids (Brown *et al*, 2002; Mihalek *et al*, 1999, 2001; Stell *et al*, 2003; Wohlfarth *et al*, 2002). This may account for the abnormal behavior of patients with 1q36 deletion, which includes the human $GABA_A$ δ-subunit gene (Windpassinger *et al*, 2002).

Another site for DHEA, as an antidepressant, is sigma-1 receptors (σ1Rs), which are unique transmitter receptors in the central system. σ1R ligands exert potent neuromodulation on excitatory neurotransmitter systems, such as the glutamate and dopamine systems, which are both involved in behavioral despair (Belozertseva *et al*, 2007; Gershon *et al*, 2007). Selective σ1R agonists cause antidepressant-like effects on rats in swim tests (Gudelsky, 1995; Urani *et al*, 2001), noting that DHEA and DHEAS are also potent σ1R agonists (Urani *et al*, 2001).

Flinders sensitive line (FSL) rats are an established genetic animal model of depression with high face, construct, and predictive validities. These rats exhibit behavioral features characteristic of depression in association with changes in the mesolimbic dopaminergic and serotonergic systems. Chronic, but not acute, treatment of the FSL rats with various antidepressants abrogates their behavioral manifestations of depression (for review, see (Overstreet *et al*, 2005; Yadid *et al*, 2000).

MATERIALS AND METHODS

In the present study, we used combined methods to elucidate the mechanism of action of DHEA as an antidepressant. Using FSL rats, we initially examined whether clinically observed correlation between depression and DHEA also occurs in a rat model for depressive-like behavior. Our premise was that finding such correlation in FSL rats would facilitate determination of the antidepressive efficiency of DHEA by its external application. Then, to suggest a mechanism of action for DHEA, as a putative anti-depressant agent, we first measured transcript levels of the σ1Rs and $GABA_A$ δ-subunit in brains of naive FSL and SD rats. Subsequently, we pharmacologically manipulated with receptor activation and measured behavior manifestation. Finally, we located its effect by perfusing DHEAS directly into selected brain sites (VTA and NAc) using mini-pumps and monitoring its longitudinal effect on behavior.

Animals

Male FSL and Sprague–Dawley (SD) rats (230–260 g) were housed two per cage under SPF conditions of constant temperature (22°C) and humidity (50%), with a 12 : 12 h dark/light cycle. Food and water were provided *ad libitum*. All animal procedures were approved by the Animal Care Committee of Bar-Ilan University and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Pharmaceutical Agents

DHEA (Research Biochemicals International; RBI, 1 Strathmore Road, Natick, MA 01760 USA) was dissolved in 0.2 ml DMSO and then diluted in saline (Khisti *et al*, 2000). DHEA was injected (0.5, 1, or 2 mg/kg for 14 days, i.p.) with or without the GABAA agonist muscimol (0.5 mg/kg for 14 days i.p.; RBI) diluted in saline. DHEAS (Sigma-Aldrich; Rehovot, Israel) was dissolved in phosphate-buffered saline (PBS) to yield 3 and 30 nM solutions.

Chronic Perfusion of Rat Brains with DHEAS

Anesthetized rats (0.5 ml ketamine and xilasine; 2 : 1 ratio) were placed in a stereotaxic apparatus, a burr hole drilled through their skull according to stereotaxic coordinates, and a 30 G cannula was lowered into the brain area of interest: VTA (4.8 mm posterior and 1 mm lateral to the bregma, and 8.4 mm ventral to the dura), NAc shell (1.6 mm anterior and 1 mm lateral to the bregma, and 7.8 mm ventral to the dura), Dorsal striatum (1.2 mm anterior and 2.4 mm lateral to the bregma, and 5 mm ventral to the dura). An ALZET osmotic pump (Durect corporation, Cupertino, CA; filled with 3 or 30 nM DHEAS or PBS) was connected to the cannula using a short tubule. At the conclusion of the behavioral tests, rats were anesthetized (xylazine 10 mg/kg + ketamine 100 mg/kg IP) and trypan blue (1 μl) was injected through the cannula into the brain. Rats were killed by decapitation and their brains were removed, fixed in formalin, and sectioned to confirm the placement of the cannula. The position of the cannula was validated by microscopy. Only data from confirmed localization of the cannula were included.

Swim Test for Measuring the Antidepressant Effect of Neurosteroids

Rats were placed in a cylindrical tank (height of 40 cm, diameter of 18 cm) that contained just enough water (25°C) so that the rat could not touch the bottom with its hind paws. The amount of time that each rat was immobile during a 5 min period was recorded. The 15-min pretest interval used to induce immobility during the standard forced swim test was omitted, as FSL rats are already highly immobile. All tests were conducted 24 h after the last drug injection and 1 h after the start of the 12 h dark period (Overstreet, 1986; Overstreet *et al*, 1988, 1992, 1994).

Extraction of Brain Tissue and DHEA Radioimmunoassay

After decapitation, brains of naive rats were removed and cut into 1 mm slices using a rat brain mold on ice. Brain regions were identified in slices by anatomical markers, and micropunches (0.5 μm) were taken and frozen in −70°C until extracted. Brain punches were extracted in absolute ethanol. Aliquots (200 μl) of the resulting ethanol solutions were completely evaporated and subjected to a commercially available DHEA radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, TX (Doron *et al*, 2006; Maayan *et al*, 2000). This RIA utilizes DHEA-DSL 9000 Active™ coated tubes.

Brain Levels of $GABA_A$ δ-Subunit and σ1R mRNA

Total RNA was isolated from brain punches of two naive groups of rats (FSL and SD) by the single-step method. Briefly, brains were punched and cDNA was synthesized. First-strand cDNA (2 μl) was added to the PCR mixture containing: 0.2 mM dNTP mix, 1 mM of each oligonucleotide primer, and 2.5 U Taq DNA polymerase (Boehringer-Mannheim, Mannheim, Germany) in the buffer supplied by the enzyme manufacturer. The following primers were used in this study.

$GABA_A$δ-subunit: cDNA sequence gene bank no. M35162, 5′GACTACGTGGGCTCCAACCTGGA3′ and 5′ACTGTGGAG GTGATGCGGATGCT3′

σ1R: cDNA sequence gene bank no. AF067769, 5′-CGG GGTGTTATTCCGT-3′ and 5′-CTCATTGCTCCCCAAG-3′

β-actin: cDNA sequence gene bank no. NM 031144, 3′-GG TATGGGTCAGAAGGACTCC-5′ and 5′-TCAGGATCTTCAT GAGGTAGTC-3′.

PCR was performed using a thermal cycler (MJ Research; Watertown, MA), after optimal conditions for detection of each transcript had been evaluated. Quantification of transcript levels were measured using 'Quantity one' software (Bio-rad laboratories; Hercules, CA, USA). Transcript levels were normalized to β-actin levels of the same sample. The data are presented as the ratio between the levels in FSL and SD rats.

DHEA and mRNA levels were measured in different groups of animals.

Statistical Analysis

Brain DHEA levels were analyzed by ANOVA 2×6 (strain × region). Behavioral data were analyzed using: one-way ANOVA for DHEA dose–response, ANOVA 2×2 for treatment with DHEA/saline with/without muscimol, or two-way ANOVA (region × treatment, in intra-brain treatments). All ANOVA analyses were followed by Scheffe-paired comparison analysis and/or Simple effect analysis. MANOVA ($2 \times 3 \times 2$) with repeated measurements (region × treatment × time) was performed to analyze the lasting effect of DHEAS.

mRNA data were analyzed using Student's *t*-test.

Data are expressed as means ± SEM, with a probability value of $p < 0.05$ being considered significant.

RESULTS

DHEA Levels in Rat Brains

We tested DHEA levels in different brain regions (amygdala, CA1 of the hippocampus, hypothalamus, NAc, Prefrontal-cortex, and VTA) of FSL and SD rats. ANOVA 2×6 (strain × region) revealed significant differences between strains ($F(1, 38) = 17.36$, $p < 0.01$, $Eta^2 = 0.31$) and regions ($F(5, 38) = 21.64$, $p < 0.001$, $Eta^2 = 0.74$). The mean of DHEA levels across all brain regions assessed in naive FSL rats were lower ($M = 689$ pmol/μg protein, SD = 65) than those measured in SD rats ($M = 1074$ pmol/μg protein, SD = 65). In addition, across strains, Scheffe analysis showed that DHEA levels in the NAc and Prefrontal-cortex were significantly lower than those in the amygdala and CA1 and that DHEA levels in the VTA were significantly lower than CA1 (Figure 1). However, no significant interaction of strain × region was found ($F(5, 38) = 1.17$, $p > 0.05$).

Measuring the Antidepressive Effect of DHEA on Immobility in the Swim-test

After finding lower levels of DHEA in limbic areas relevant to depression of FSL rats, we further tested DHEA as a possible antidepressant by adding it exogenously. The performance of FSL rats in the swim test was measured 24 h after the last of 14 daily i.p. injections of DHEA (0.5, 1, or 2 mg/kg) or saline. One-way ANOVA revealed a significant difference in immobility time between dose-

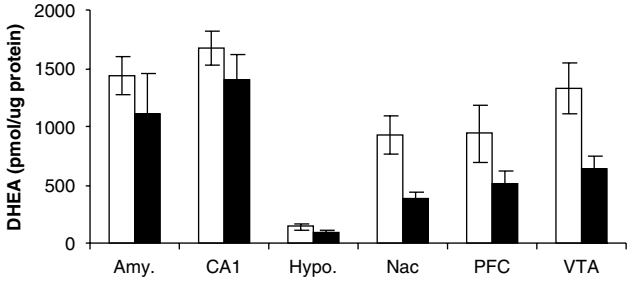

Figure 1 DHEA levels in brain regions of naive rats. DHEA levels were measured by radioimmunoassay in brain regions of FSL (solid column) and SD rats (empty column). The mean ± SEM is presented. Amy-amygdala ($n = 4$ and 3 for SD and FSL respectively), CA1 ($n = 4$ and 5 for SD and FSL respectively), Hypo—hypothalamus ($n = 6$ and 4 for SD and FSL respectively), Nac—nucleus accumbens ($n = 4$ and 5 for SD and FSL respectively), PFC—prefrontal cortex ($n = 3$), VTA—ventral tegmental area ($n = 4$ and 5 for SD and FSL respectively).

groups ($F(3,18)=5.05$, $p<0.01$, $Eta^2=0.46$). Paired comparison tests according to Scheffe revealed a significant difference between the saline treatment ($M=170$ s, SD = 43) *vs* treatment with 1 mg/kg DHEA ($M=96$ s, SD = 24), but not *vs* treatment with 0.5 or 2 mg/kg DHEA ($M=124$ s, SD = 41 and Mean = 136 s, SD = 15, respectively) (Figure 2). Moreover, we found no significant effect of DHEA (1 mg/Kg/14 days) on the immobility time of control SD rats (data not shown).

Brain Levels of $GABA_A$ δ-Subunit and σ1R mRNA

To elucidate the mechanism of action of DHEA as an antidepressant, we measured the levels of mRNA for the $GABA_A$ δ-subunit and σ1R in brain regions reported to play a role in depression (Overstreet *et al*, 2005). In the VTA of FSL rats, the levels of transcripts of the $GABA_A$ δ-subunit were significantly higher than in SD rats, whereas in the NAc of FSL rats they were significantly lower than in SD rats (*T*-test, $p<0.05$, Figure 3). No significant changes in the transcript levels of the $GABA_A$ δ-subunit were found in the PFC, CA1, and amygdala (data not shown). Also, no significant differences were seen between the levels of σ1R transcripts in FSL and SD rats in all tested brain regions.

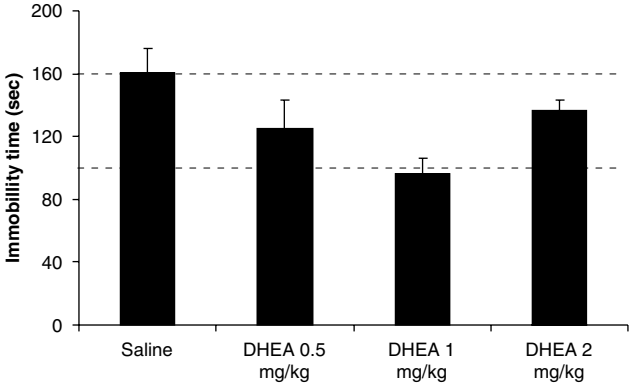

Figure 2 Performance of DHEA-treated FSL rats in the swim test. FSL rats were injected (i.p.) with DHEA (0.5, 1, or 2 mg/kg) or saline for 14 days and were subjected to the swim test 24 h after the last injection. Lower dashed line indicates the average immobility time of naive SD rats. Upper dashed line indicates the average immobility time of naive FSL rats. Mean ± SEM of immobility time (sec) is presented. $N=7$ for saline, $n=6$ for dose of 1 mg/kg DHEA and $n=5$ for doses of 0.5 and 2 mg/kg DHEA.

GABA-Ergic Modulation of the Antidepressant Effect of DHEA

As DHEA is a negative modulator of $GABA_A$Rs, we examined whether a $GABA_A$ agonist, muscimol, can block the anti-depressant activity of DHEA. ANOVA 2 × 2 (DHEA (1 mg/kg) or saline × muscimol (0, 0.5 mg/kg)) shows a significant effect of DHEA compared to saline ($F(1,21)=12.88$, $p<0.01$, $Eta^2=0.38$) and a significant interaction ($F(1,21)=1.21$, $p<0.05$, $Eta^2=0.18$; Figure 4). Simple effect analysis to test the source of interaction revealed a significant difference in the DHEA-treated groups with/without muscimol ($F(1,10)=7.24$, $p<0.05$, $Eta^2=0.42$). Rats preinjected with muscimol 30 min before treatment with DHEA were significantly more immobile ($M=138$ s, SD = 11) than rats treated with DHEA alone ($M=96$ s, SD = 24). Simple effect analysis did not find a significant effect of muscimol on saline-treated rats ($M=157$ s, SD = 8, $M=170$ s, SD = 43, respectively; $F(1,11)=0.44$, $p>0.05$). Thus, treatment with muscimol can reverse the antidepressant action of DHEA.

Effects of Intra-brain Perfusion of DHEAS on Depressive Behavior

As we found alterations in both the levels of DHEA and δ subunit in the VTA-NAc, we assumed that the effect of exogenous DHEA on depressive behavior is mediated by these regions. FSL rats were perfused with a soluble derivate

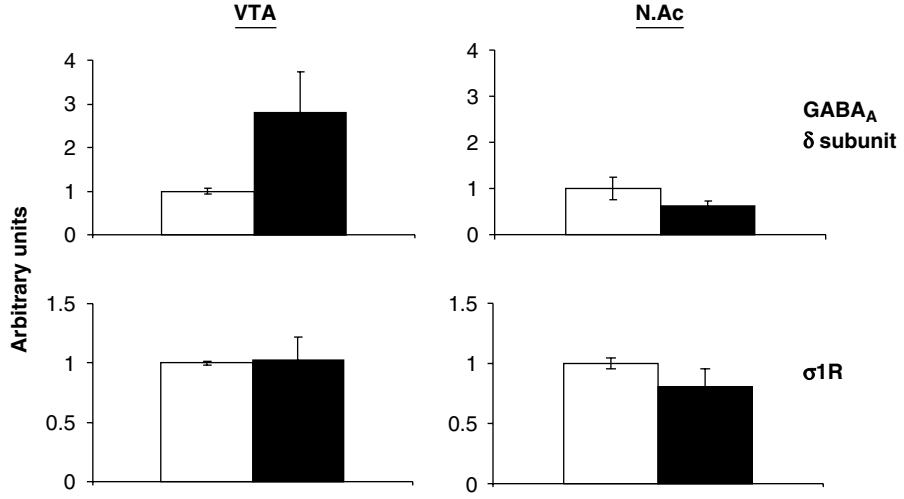

Figure 3 Levels of mRNA transcripts for $GABA_A$ δ-subunit and σ1R in the VTA and NAc regions of FSL and SD rat brains. Transcript levels are presented as arbitrary units ($GABA_A\delta$-subunit/β actin normalized to SD, and σ1R/β actin normalized to SD). FSL rats (solid columns), SD rats (empty columns). The mean ± SEM is presented. $GABA_A$ δ-subunit, $n=6$, except for the VTA region of FSL rats ($n=4$); σ1R, $n=5$ for VTA and $n=7$ for NAc.

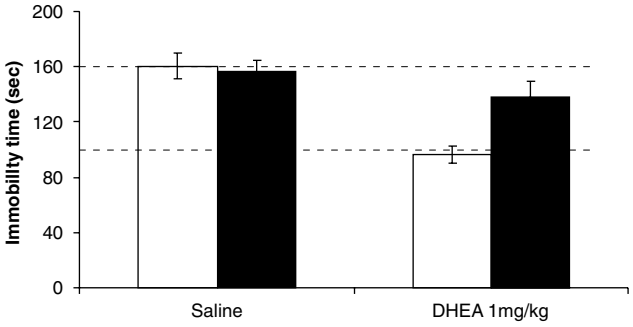

Figure 4 Immobility of saline, DHEA, and muscimol-treated FSL rats in the swim test. FSL rats were injected daily (i.p.) with saline or DHEA (1 mg/kg) with (black columns) or without muscimol (0.5 mg/kg, white columns) for 14 days. After which they were subjected to swim tests. Note: the data for 1 mg/kg DHEA treated animals is the same presented in Figure 2. Mean ± SEM of immobility time (sec) is presented. $N=7$ for treatment with saline without muscimol and $n=6$ for all other groups. Lower dashed line indicates the average immobility time of naive SD rats. Upper dashed line indicates the average immobility time of naive FSL rats.

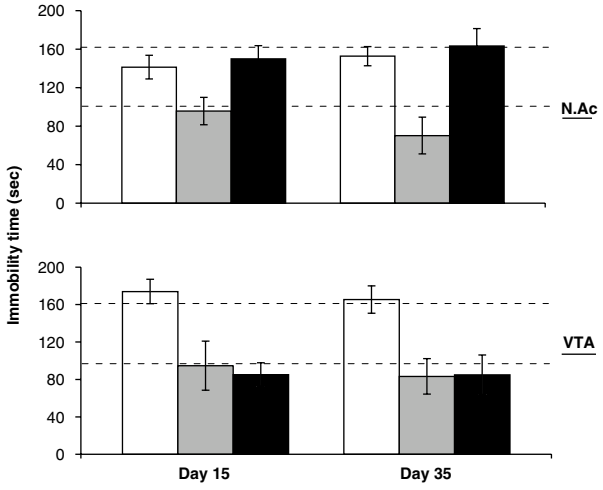

Figure 5 Immobility in swim tests of FSL rats after direct application of DHEAS into the VTA or NAc. FSL rats were perfused with DHEAS (3 nM (gray columns); 30 nM (black columns)) or PBS (white columns) directly into their VTA or NAc for 14 days. One day (day 15 of the experiment) and 21 days (day 35 of experiment) after the completion of the treatment rats were subjected to swim tests. Lower dashed line indicates the average immobility time of naive SD rats. Upper dashed line indicates the average immobility time of naive FSL rats. Mean ± SEM of immobility time is presented. Day 15: PBS, VTA ($n=7$), NAc ($n=6$); 3 nM DHEAS, VTA ($n=6$), NAc ($n=6$); 30 nM DHEA, VTA ($n=7$), NAc ($n=6$). Day 35: PBS, VTA ($n=5$), NAc ($n=6$); 3 nM DHEAS, VTA ($n=5$), NAc ($n=4$); 30 nM DHEA, VTA ($n=4$), NAc ($n=5$).

of DHEA (DHEAS, 3 or 30 nM) or vehicle (PBS) into the VTA and NAc.

The immobility time during the swim test 24 h after the completion of 14 days, treatment is presented in Figure 5. ANOVA 2×3 (region × treatment) revealed a significant difference ($F(2,32)=11.04$, $p<0.01$, $Eta^2=0.41$) between the three treatments (3 or 30 nM DHEA, or PBS) and a significant interaction (region × treatment; $F(2,32)=6.93$, $p<0.01$, $Eta^2=0.3$).

A Simple effect analysis indicated a significant difference between regions according to dose. Whereas both doses of DHEAS in the VTA significantly ($F(2,17)=13.74$, $p<0.01$, $Eta^2=0.62$) lowered immobility time (($M=95$ s, SD = 26 for 3 nM and $M=85$ s, SD = 13 for 30 nM; compared to treatment with PBS ($M=174$ s, SD = 13)), in the NAc, only treatment with 3 nM DHEAS significantly ($F(2,15)=4.71$, $p<0.05$, $Eta^2=0.39$) affected the rat's performance (($M=96$ s, SD = 14) compared to PBS ($M=141$ s, SD = 12)).

We also tested the long-term effect of DHEAS treatment on immobility of FSL rats. The same rats (treated with PBS or DHEAS (3 or 30 nM)) were again given the swim test 21 days after the cessation of treatment (day 35 of experiment). MANOVA $2\times3\times2$ (region × treatment × time) with repeated measures have not found a significant difference according to time ($F(1,23)=1.52$, $p>0.05$). Moreover, we did not find significant interactions of time with area and/or treatment. Thus, the significant effect of intra-brain DHEAS continues for at least 21 days after completion of treatment.

We further tested the brain region's specificity of the DHEAS treatment. We perfused DHEAS or a vehicle (PBS) into the dorsal striatum of FSL rats, an area outside the mesolimbic pathway. We used 3 nM DHEAS, the dose that had a beneficial effect when applied both into the VTA and NAc. After 14 days of treatment, the rat's performance in the swim test was monitored and compared to the above mentioned treatments in both the NAc and VTA. ANOVA 3×2 (region (NAc, VTA, dorsal striatum) × treatment (DHEA, PBS)) revealed significant differences according to treatment ($F(1,27)=8.78$, $p<0.01$, $Eta^2=0.25$) and a significant region × treatment interaction ($F(2,27)=4.89$, $p<0.05$, $Eta^2=0.27$). Simple effect analysis did not find any significance between the different treatments in the dorsal striatum ($M=136$ s, SD = 15 for PBS or M = 152 s, SD = 21 DHEAS 3 nM; $F(1,6)=0.44$, $p>0.05$). Thus, DHEAS has no antidepressive effect when applied outside the NAc-VTA circuit.

DISCUSSION

Although the usefulness of DHEA as an antidepressant has been suggested (Dubrovsky, 2005; Wolf and Kirschbaum, 1999; Wolkowitz *et al*, 1999), its mechanism of action and role in depression is poorly understood. Herein, we used an animal model of depression (FSL rats) to demonstrate alterations in DHEA levels in some brain regions relevant to depression. This is the first time that decreases in DHEA levels have been shown in brain regions central to manifestation of depressive behavior in an animal model (Figure 1). Furthermore, chronic treatment of FSL rats with DHEA significantly decreased their immobility in the swim test (Figure 2) to the same extent as conservative antidepressants (Dremencov *et al*, 2004), thus confirming an antidepressive-like action of DHEA in an animal model of depression. The dose response curve of DHEA is concordant with other reports (Urani *et al*, 2001) demonstrating a narrow therapeutic window.

DHEA may act through the neurotransmitter receptors $GABA_AR$, $\sigma1R$ and NMDAR (Majewska *et al*, 1990; Monnet *et al*, 1995; Urani *et al*, 2001). A role in depression for $\sigma1R$

and $GABA_AR$ has already been suggested (Gudelsky, 1995; Lloyd *et al*, 1989; Urani *et al*, 2001). Here (Figure 3) we demonstrate that FSL rats do not express differences in the levels of σ1R mRNA, but that they do so in those of δ-subunit of the $GABA_AR$ (compared to SD rats) in the VTA-NAc (Figure 3). These mesolimbic regions were suggested to have a central role in depressive behavior (Overstreet *et al*, 2005). Incorporation of the δ-subunit dramatically augments the GABA-enhancing actions of the steroid (Belelli *et al*, 2002; Mihalek *et al*, 1999, 2001). Therefore, the elevated levels of mRNA for the $GABA_A$ δ-subunit in the VTA of FSL rats might be due to an intrinsic GABA-ergic intraneuron modulation (compensation) for low levels of DHEA in this brain region (Concas *et al*, 1999; Gulinello *et al*, 2001; Holt *et al*, 1996; Mahmoudi *et al*, 1997). The contrasting lower levels of mRNA for the δ-subunit in the NAc of FSL compared to SD rats can be explained by different localization of GABA modulation on ascending neurons (Akiyama *et al*, 2004; Bankson and Yamamoto, 2004; Tao and Auerbach, 2002; Yan *et al*, 2004). Our results may point to a different role for the $GABA_ARs$ in the NAc compared to the VTA, some of which may be explained by localization of the receptors pre- or post-synaptically (Ikemoto *et al*, 1997; Yan *et al*, 2004). Moreover, our results suggest that an imbalance in the mesolimbic GABA-ergic system together with the alterations in DHEA levels may lead to the pathological behavioral outcomes observed in the FSL rats.

The GABA-ergic system is an important regulator of dopaminergic neurons (Akiyama *et al*, 2004), which are relevant to depression (Dremencov *et al*, 2006; Friedman *et al*, 2007). Specifically, $GABA_ARs$ within the VTA regulate dopamine output to the NAc (Bankson and Yamamoto, 2004; Yan *et al*, 2004), suggesting a modulatory role for these receptors in depression (Garcia-Alloza *et al*, 2006). Furthermore, co-administration of a $GABA_A$ agonist (muscimol) and DHEA blocked the beneficial effect of DHEA on depressive behavior (Figure 4), confirming that the $GABA_AR$ is the main target for DHEA in depression. Hence, elevating mesolimbic DHEA levels exogenously may overcome lower endogenic DHEA levels by activating the GABAergic system and correcting depressive-like behavior. It is important to note that DHEA is a negative modulator of the $GABA_AR$. Unlike full antagonists, such as bicuculline, which may trigger seizures, it does not have aversive side effects. This may explain the nonavailability of full $GABA_AR$ antagonists as antidepressants. For the same reason, we avoided manipulating with $GABA_AR$ antagonists to augment DHEA antidepressive effects. Although we found a central role for $GABA_AR$ in the antidepressive mechanism of DHEA, but did not find differences in σ1R transcript levels in FSL compared to SD rats, we do not rule out the possibility that other receptors such as σ1Rs and NMDARs may also be involved in DHEA antidepressive effects.

The content of monoamines in the limbic regions of FSL rats is not affected by repeated exogenously applied DHEA (unpublished findings, R Genud and G Yadid) in contrast to treatment with conservative anti-depressants (Overstreet *et al*, 2005; Zangen *et al*, 1997), which further suggests that DHEA may affect depressive behavior differently than the established mechanism for antidepressants (Shapira *et al*, 1994).

To locate the DHEA site of action in the brain, we treated FSL rats with DHEAS directly into the brain. DHEAS is more soluble in water than DHEA, is converted to DHEA in the cerebral tissue (Kroboth *et al*, 1999), and is reported to affect the brain neurotransmitter receptors ($GABA_AR$, NMDAR, σ1R) in the same mode as DHEA (Majewska, 1992; Monnet *et al*, 1995; Urani *et al*, 2001). Therefore, DHEAS was used for the direct intrabrain administration. In FSL rats, perfusion of DHEAS directly into the VTA and Nac, but not into the dorsal striatum, caused the same effects on depressive behavior as i.p. injection of DHEA (Figure 5). These results suggest that the antidepressive action of DHEAS in the brain is localized in the mesolimbic system. In the NAc, the sensitivity of $GABA_AR$ to DHEAS might be higher, because as the concentration of DHEAS increased, its effect on depressive behavior decreased. This sensitivity can be explained by our neurochemical findings indicating lower DHEA levels in the NAc compared to the VTA in both SD and FSL rats (Figure 1). Thus, $GABA_ARs$ are tonically exposed in the NAc to low concentration of local DHEA compared to the $GABA_ARs$ in the VTA. In addition, we found a decrease in the levels of the $GABA_A$ δ-subunit in the Nac, whereas there is an increase in the VTA of FSL rats compared to SD rats. These lowered levels in the NAc can explain a higher sensitivity for DHEAS in the NAc compared to the VTA. Moreover, this is reminiscent of high doses of progesterone downregulating $GABA_AR$ in mice (Czlonkowska *et al*, 2001), due to excessive stimulation of $GABA_ARs$ by progesterone metabolites (neurosteroids).

The effect of DHEAS on the behavioral manifestations of depression persisted for at least 21 days after completion of the 14-day treatment (Figure 5), indicating its long-lasting effect. This long-lasting effect of DHEAS might either be due to changes in $GABA_ARs$ density or sensitivity or to changes in the neurosteroid metabolism and synthesis in the brain.

On the basis of our results, we propose a role for the neurosteroid DHEA/DHEAS in the pathophysiology and treatment of depression. We found that DHEA/DHEAS acts as an antidepressant via the GABA-ergic mechanism, unlike the currently used antidepressants that target monoamine systems, and has a long-term effect on the mesolimbic VTA-NAc pathway, which is centrally involved in depression.

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DISCLOSURE/CONFLICT OF INTEREST

We declare that except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and that there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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