Effects of dehydroepiandrosterone in amphetamine-induced schizophrenia models in mice

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ABSTRACT

Objective: To examine the effects of dehydroepiandrostrosterone (DHEA) on animal models of schizophrenia.

Methods: Seventy Swiss albino female mice (25-35 g) were divided into 4 groups: amphetamine-free (control), amphetamine, 50, and 100 mg/kg DHEA. The DHEA was administered intraperitoneally (ip) for 5 days. Amphetamine (3 mg/kg ip) induced hyperlocomotion, apomorphine (1.5 mg/kg sc) induced climbing, and haloperidol (1.5 mg/kg sc) induced catalepsy tests were used as animal models of schizophrenia. The study was conducted at the Animal Experiment Laboratories, Department of Pharmacology, Medical School, Eskisehir Osmangazi University, Eskisehir, Turkey between March and May 2012. Statistical analysis was carried out using Kruskal-Wallis test for hyperlocomotion, and one-way ANOVA for climbing and catalepsy tests.

Results: In the amphetamine-induced locomotion test, there were significant increases in all movements compared with the amphetamine-free group. Both DHEA 50 mg/kg (p<0.05), and 100 mg/kg (p<0.01) significantly decreased all movements compared with the amphetamine-induced locomotion group. There was a significant difference between groups in the haloperidol-induced catalepsy test (p<0.05). There was no significant difference between groups in terms of total climbing time in the apomorphine-induced climbing test (p>0.05).

Conclusion: We observed that DHEA reduced locomotor activity and increased catalepsy at both doses, while it had no effect on climbing behavior. We suggest that DHEA displays typical neuroleptic-like effects, and may be used in the treatment of schizophrenia.

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Schizophrenia is characterized by disorders in perception and affect, disorganized behaviors and speech, cognitive deficiencies, and decline in psychosocial functioning. In most cases, the disease has a slowly developing onset after a period of social withdrawal, decrease in interest, reduced self-care, cognitive, and behavioral alterations.\(^1\)

Dehydroepiandrosterone (DHEA) and its sulfate ester dehydroepiandrosterone sulfate (DHEAS) are major secreted hormones of the human adrenal cortex. Dehydroepiandrosterone, a 19 carbon steroidal compound, is synthesized from cholesterol and is the source of all steroidal hormones in humans.\(^2\) The course of schizophrenia exhibits gender differences, which suggest the involvement of gender specific hormones in the disease. Estrogen is especially thought to play a preventive role in schizophrenia, as the disease begins at later ages in females compared with males. This led researchers to perform clinical studies on the effect of DHEA, an estrogen precursor, in schizophrenia. These findings can be interpreted as further evidence for a protective effect of estrogens in schizophrenia, possibly due to the known antidopaminergic activities of these hormones.\(^3\) In addition, it was reported that DHEA modulated dopamine receptor activation, and it was hypothesized that both DHEA and DHEAS inhibited dopamine D2 receptor signalization.\(^4\) Some studies suggested that DHEA enhanced dopamine release via inhibition of presynaptic dopamine receptors, while it alleviated locomotor activity by blocking postsynaptic D2 receptors and showed activity resembling typical antipsychotics.\(^5,6\) In this study we aimed to investigate the possible antipsychotic and protective effects of DHEA at different doses on experimental schizophrenia models in mice.

**Methods.** **Animals.** Seventy Swiss albino mice (25-35 g) were used in the study and sheltered in standard conditions of light (12 hours light/dark cycle) and temperature (21±1°C). Food and water were available ad libitum. Experiments were performed in a sound isolated psychopharmacology laboratory in the Department of Pharmacology, Eskisehir Osmangazi University, Eskisehir, Turkey between 08:00-14:00 hours with the permission of the local Ethical Committee of Eskisehir Osmangazi University Medical School for the care and use of laboratory animals in line with the Declaration of Helsinki. The study was conducted between March and May 2012.

**Drugs.** Dehydroepiandrosterone (Sigma, St. Louis, MO, USA) was used at doses of 50 and 100 mg/kg and was dissolved in a solution of saline and DMSO 1:10, and was administered intraperitoneally (ip). Haloperidol (Aris, Istanbul, Turkey) (1.5 mg/kg subcutaneously [sc]), amphetamine (Sigma, St. Louis, MO, USA) (3 mg/kg ip), and apomorphine HCl (1.5 mg /kg sc) (Sigma, St. Louis, MO, USA) were dissolved in the same solution.

**Experimental groups and study design.** Animals were randomly divided into 3 groups as follows: Group 1: control group (n:28): a solution of saline and DMSO 1:10 was administered ip for 5 days. Group 1a (n:7): tested with amphetamine-induced hyper locomotion. Group 1b (n:7): tested with apomorphine-induced climbing. Group 1c (n:7): tested with haloperidol-induced catalepsy. Group 1d (n:7): tested with spontaneous locomotor activity.

Group 2 (n:21): 50 mg/kg DHEA was administered ip for 5 days. Group 2a (n:7): tested with amphetamine-induced hyper locomotion. Group 2b (n:7): tested with apomorphine-induced climbing. Group 2c (n:7): tested with haloperidol-induced catalepsy.

Group 3 (n:21): 100 mg/kg DHEA was administered ip for 5 days. Group 3a (n:7): tested with amphetamine-induced hyper locomotion. Group 3b (n:7): tested with apomorphine-induced climbing. Group 3c (n:7): tested with haloperidol-induced catalepsy.

**Experimental schizophrenia models.** Three different experimental models were used: a) Amphetamine-induced hyper locomotion test: one hour after the administration of DHEA or vehicle, 3 mg/kg amphetamine was injected ip. Then the stereotypical, ambulatory, horizontal, total movements, and the distance travelled were recorded every 30 minutes for 5 minutes for a total 3 hour period by an automatic video-computerized system (MAY AMS 02 animal activity monitoring sistem, COMMAT, Ankara, Turkey).\(^7\) b) Apomorphine-induced climbing test: 1.5 mg/kg apomorphine was injected sc one hour after the administration of DHEA or vehicle and mice were immediately placed in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm). The total and maximum climbing times on the inside of the cage were recorded for 30 minutes.\(^8\) c) Haloperidol-induced catalepsy test: 1.5 mg/kg haloperidol was injected sc one hour after the administration of DHEA or vehicle, and the mice were observed in the first, second, and the third hours after haloperidol injection for 5 minutes in each hour. Mice were positioned with

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both front limbs on a 4 cm high bar and the total time that the mice were kept in this position was recorded for a maximum period of 300 seconds.9,10

**Statistical analysis.** The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 19.0 program was used for the analysis of the data of hyperlocomotion test and results were expressed as median and 25-75% percentile. Kruskal-Wallis test was used for abnormally distributed data and multiple comparisons of this test were made using Dunn’s method. A value of $p<0.05$ was accepted as statistically significant. The SPSS 19.0 and SigmaStat 3.5 programmes were applied to analyze the results of the climbing and catalepsy tests. The results were given as mean and SEM. One-way ANOVA test was used for the data of climbing and catalepsy tests, and the Student-Newman-Keuls method was used for multiple comparisons.

**Results.** **Amphetamine-induced hyperlocomotion test.** Stereotypical, horizontal, ambulatory, total movements, and the distance travelled were recorded. In the amphetamine-induced locomotion group, there were significant increases in all movements (stereotypical, horizontal, ambulatory, total movements, and the distance travelled) compared with the amphetamine-free group ($p<0.05$). Dehydroepiandrosterone 50 mg/kg ($p<0.05$), and DHEA 100 mg/kg ($p<0.01$) significantly decreased all movements compared with the amphetamine-induced locomotion group (Figures 1a, 1b, 1c, 1d, & 1e). In this test, locomotor activity was recorded for 5 minutes in every 30 minutes over a 3 hour period. Considering the results of all movements in terms of time points recorded, there was no significant difference between the DHEA 100 mg/kg group, and the amphetamine-free control group ($p>0.05$). The results are shown in Figures 1a, 1b, 1c, 1d, & 1e.

**Catalepsy test.** Haloperidol-induced catalepsy time was recorded 1, 2, and the 3 hours after drug or vehicle injection for 5 minutes in each hour. There was a significant difference between groups in the catalepsy time records of all the 3 hour observations. The difference was dose-independent (Figure 2).

**Apomorphine-induced climbing test.** There was no significant difference between groups in terms of total climbing time ($p>0.05$) (Figure 3).

**Discussion.** Dehydroepiandrosterone and its sulfate ester DHEAS, synthesized in the ovary, testes, and brain, are the most important of the neuro steroids. These are the main products of the adrenal gland and are the precursors of androgenic and estrogenic steroids.11,12

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**Figure 1 -** The effects of dehydroepiandrosterone (DHEA) on locomotor activity. A) Stereotypical movement numbers compared with time in all groups. B) The number of ambulatory movements compared with time in all groups. C) The number of total movements compared with time in all groups. D) The number of horizontal movements compared with time in all groups. E) Distance compared with time in all groups. *$p<0.05$ compared with the amphetamine group; +$p<0.05$ compared with the control group; **$p<0.01$ compared with the amphetamine group, ++$p<0.01$ compared with the control group; n:7 mice for each group.
It is known that DHEA has effects on neuronal growth,13 memory and cognitive functions, anxiolytic and antidepressant, 14,15 and immunoprotective effects.12 As a result of the experimental and clinical studies, taking into account the neuroprotective and neuropsychopharmacological effects, DHEA is thought to be associated with the pathophysiology of schizophrenia.16

In light of the results of many studies, dopamine is known to play an important role in the pathophysiology of schizophrenia. It is known that an increase in dopaminergic activity in the mesolimbic pathway is associated with positive symptoms while a reduction in dopaminergic activity in the mesocortical pathway is related to negative symptoms of schizophrenia.17,18 Psychostimulant substances with dopamine agonist activity such as amphetamine induce dopaminergic activity and generate an increase in locomotor activity resembling psychosis.5,19

In this study, we found that both 50 and 100 mg/kg doses of DHEA decreased the number of movements and the distance travelled compared with controls in the amphetamine-induced hyperlocomotion test. We also observed that in the apomorphine-induced climbing test, DHEA at both doses made no alteration in total climbing time. In addition, catalepsy time was increased at both doses of DHEA in the second and the third hour records of the haloperidol-induced catalepsy test.

In the present study, the findings that DHEA reduced the number of total movements and the distance travelled suggest an antidopaminergic and neuroleptic-like activity of DHEA. Perez-Neri et al20 found that DHEA affected brain monoamines, and high doses (30-200 mg/kg) of DHEA increased the dopamine turnover. On the contrary, another study21 reported that a low dose of DHEA (2 mg/kg) increased the levels of dopamine in the ventral tegmental area and decreased dopamine turnover in prefrontal cortex, hippocampus, and hypothalamus. It is also suggested that DHEA is involved in dopamine receptor modulation and both DHEA and DHEAS inhibited D2 receptor signaling.3

Dehydroepiandrosterone is the precursor of estrogen, progesterone, and testosterone. These steroidal compounds are thought to possess behavioral effects not only by genomic activity, but also by affecting ion channels and neurotransmitter transporter systems. The serotonin agonistic and D2 receptor modulatory effects of estrogen are well documented.22 Estrogens have complex effects on the dopaminergic system and are believed to act on dopaminergic neurotransmission dose and time dependently. Estrogens present neuroleptic-like effects by modulating dopamine receptor sensitivity in animals and antidopaminergic effects in humans,23 in so much that low dose hormone replacement therapy was associated with an improvement in the negative symptoms of schizophrenia.24 Further evidence for the protective role of estrogen in schizophrenia is the fact that the disease has a late onset and a second peak at ages of 40-45 in women, and females respond to antipsychotic treatment while negative symptoms are much resistant to treatment in males.25

In some behavioral studies, estrogen was reported to show dopamine receptor blocking effects and alleviate dopamine associated behaviors such as amphetamine-induced locomotion and apomorphine-induced behaviors.26 In this study, we observed that DHEA decreased locomotor activity in female mice. This finding suggests an antidopaminergic and neuroleptic effect of DHEA, which is similar to estrogen and can be explained with the estrogen precursor feature of DHEA.

Although there is not much evidence on the effects of DHEA on dopaminergic systems, the studies in which the role of estrogen on dopaminergic systems was investigated reported that estrogen modulated the mesolimbic dopaminergic pathway while it inhibited...
the meso striatal pathway. Tardive dyskinesia, akathisia, and parkinsonism are side effects of neuroleptic treatment and are seen more frequently in women. Estrogen is administered to improve tardive dyskinesia. However, in menopausal women tardive dyskinesia occurs due to sudden withdrawal of estrogen in a similar manner to dopaminergic hypersensitivity observed in sudden withdrawal of neuroleptics. Side effects noted in high estrogenic periods of women were improved with typical antipsychotics, but there was no healing in women using atypical antipsychotics. In our study, we suggest that DHEA resembles an activity like typical antipsychotics considering its reducing effect on locomotor activity and increasing effect on catalepsy time. Catalepsy is more frequent in treatment with typical neuroleptics than atypical antipsychotic treatment. In this study, we observed that 50 and 100 mg/kg doses of DHEA increased catalepsy time in haloperidol-induced catalepsy test. It is suggested that haloperidol (a typical antipsychotic) induced catalepsy is modulated via D2 receptors in the striatopallidal pathway. We suggest that DHEA exhibited a catalegetic effect via striatopallidal D2 receptors. Apomorphine contributes to climbing behavior through the striatal D2 receptors. In this study, we found that DHEA did not make any change in apomorphine-induced climbing time, so we suggest that DHEA did not affect striatal D2 receptors. Given the fact that estrogen plays a protective role in schizophrenia, we hypothesized that the estrogen precursor DHEA would also have protective effects in an experimental schizophrenia model.

In conclusion, we suggest that DHEA acts as a typical antipsychotic agent at doses of 50 and 100 mg/kg and especially has a marked protective effect at the 100 mg/kg dose in experimental schizophrenia models. However, further studies are needed taking into account the lack of current evidence.

References

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