Research article

Long-term supplementation of dehydroepiandrosterone improved depressive-like behaviors by increasing BDNF expression in the hippocampus in ovariectomized rats

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ABSTRACT

Objective: Dehydroepiandrosterone (DHEA), a precursor of estrogen, partially exhibits its biological effect after conversion to estrogen. Its biological significance in perimenopausal depressive disorder or postpartum depression remains unknown. Here, we observed the effects of long-term supplementation of DHEA on depression-like behaviors in ovariectomized rats.
Methods: We established the model as one of sex hormone deficiency in female rats by bilateral ovariectomy. We observed the effects of 13.3 mg/kg DHEA or 0.27 mg/kg estradiol were given daily by gavage for 12 weeks on lipid metabolism, glucose tolerance, and depression-like behaviors in ovariectomized rats. Furthermore, the expression of brain-derived neurotrophic factor (BDNF) and its signaling molecule in the hippocampus was analyzed.
Results: The 12-week supplementation of DHEA or estradiol significantly alleviated weight gain and improved the glucose tolerance in the ovariectomized rats. Moreover, Long-term supplement of DHEA or estradiol significantly increased sucrose preference and locomotion activities, and reduced immobility duration of the ovariectomized rats in the water. Both DHEA and estradiol treatments increased the expression of BDNF, phosphorylation of ERK and CREB, and ERβ, but not that of ERα in the hippocampus of the ovariectomized rats.
Conclusions: Overall, chronic treatment with DHEA improved depression-like behaviors in ovariectomized rats, suggesting that it may be useful for the treatment of sex hormone deficiency such as perimenopausal depressive disorder or postpartum depression.

1. Introduction

Perimenopause is the period during which the ovarian functions gradually decline, leading to fluctuations or reduction in estrogen and progesterone levels [1]. A prospective study has shown that among women without a history of depression, the risk of depression during the perimenopausal period is two times higher than that during the premenopausal period [2]. Furthermore, the risk of depression during the menopausal transition period is two to four times higher than that during the premenopausal period; the risk ratio is five times higher in women with a history of depression than in those without a history of depression [3]. Besides, hormonal changes during pregnancy and postpartum are thought to play a role in the etiology of postpartum depression (PPD) [4, 5]. A preliminary clinical study has shown that 17β-estradiol (E2) replacement effectively ameliorates perimenopausal depression [6]. Additionally, a randomized controlled clinical trial indicated that the supplementation of E2 with low-dose progesterone effectively prevents perimenopausal depression [7]. Currently, hormone replacement therapy (HRT), consisting of estrogen with or without progesterone, has been used to alleviate perimenopausal symptoms and prevent osteoporosis [8]. Although its efficacy in perimenopausal depression is well documented, estrogen is not approved by the FDA for the treatment of mood disturbances in perimenopausal women [9]. Thus, other estrogenic compounds should be considered to improve perimenopausal depression and PPD.

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Dehydroepiandrosterone (DHEA) is an inert precursor of various sex hormones such as estrogen and androgen, but it has extremely weak androgenic activity. In adult women, 70%–90% of DHEA is synthesized in the adrenal cortex, 10%–30% in the ovary, and a small portion in the brain. It exists in the form of dehydroepiandrosterone sulfate (DHEAS) in the blood. Before menopause, approximately 75% of estrogen is derived from DHEAS, and it increases to nearly 100% post menopause [10]. It has been shown that the plasma DHEAS concentration is lower in patients with depression than in those without depression [11]. DHEA plays a significant role in neuroprotection, neurogenesis, and neuronal survival, as well as apoptosis and catecholamine synthesis and secretion [12, 13]. The risk of ovarian cancer increases with higher levels of androstenedione and DHEAS, but without difference between premenopausal and postmenopausal women [14]. Supplementation of exogenous DHEA may increase the risk of breast cancer in postmenopausal women associated with pre-existing abdominal obesity [15], but not increase the risk and incidence of endometrial cancer [16]. Moreover, depression score and performance were significantly improved after DHEA supplementation in patients with depression, suggesting that DHEA may have antidepressant effects [7]. DHEA exerts its neuroprotective effect by increasing the expression of brain-derived neurotrophic factor (BDNF), acetylcholine, and catecholamine in the hippocampus of rats with vascular dementia [17]. DHEA(S) may act by transforming to more potent steroids or by activating androgen receptors or estrogen receptors (ERs) in the brain [18].

The evidence has shown that high cortisol level in depressed people, and cortisol is a well-known anti-insulinergic hormone which impairs oral glucose tolerance in major depression [19]. Meanwhile, the higher TG and lower HDL-cholesterol levels in depression [20]. In patients with depression, the expression of BDNF is significantly reduced; thus, BDNF can be used as a biomarker to monitor the condition of patients with depression [21]. The serum BDNF level also decreases, and it has independent predictive value for depression in patients with perimenopausal syndrome [22]. BDNF binds to its receptor tyrosine kinase B (TrkB) and phosphorylates TrkB, which activates multiple downstream signaling pathways. Protein kinase C via the phospholipase Cγ pathway can affect synaptic plasticity. The phosphatidylinositol 3-kinase pathway activates protein kinase B, which affects cell survival. Besides, downstream effectors via the mitogen-activated protein kinase or ERK pathway influence cell growth and differentiation [23].

The biological action of DHEA in neuroprotection has been recognized, but its role in the pathogenesis of perimenopausal depression and PPD, and mechanism of action remain unclear. In the present study, we evaluated the effects of long-term supplementation of DHEA on glucose and lipid metabolism, the depressive-like behaviors and analyzed the expression of BDNF, phosphorylation of ERK and CREB, ERα and ERβ in the hippocampus of ovariectomized rats.

2. Materials and methods

2.1. Animals

Eight-week-old, pathogen-free female Sprague–Dawley rats (n = 32) were purchased from Experimental Animal Center of Zhejiang Province (license number: SCXK (Zhejiang) 2014-0001). They were reared in a specific-pathogen free animal room in the Experimental Animal Center of Ningbo University School of Medicine, China under the following controlled conditions: 12-:12-h dark/light cycle, room temperature of 22 °C ± 1 °C, and humidity of 55% ± 5%. The four rats were housed in one cage, only sucrose preference test, each rat was housed in a single cage. The experimental procedures were approved by the Ethics Committee of Laboratory Animal Use and Care of Ningbo University. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (eighth edition).

2.2. Bilateral ovarian ablation surgery

After 1 week of acclimatization, bilateral ovarian ablation was performed in half of the rats, and the remaining rats were subjected to a sham operation. Bilateral ovarian ablation was performed in an animal operating room with the rats under anesthesia induced by intraperitoneal injection of 10% chloral hydrate. Subsequently, the rats were depilated, and an incision was made in the lower third of the abdomen to locate the uterus, which was Y-shaped. We then removed both ovaries and sutured the incision. For the sham control group, the operation was the same as the bilateral ovarian ablation, but only fat around the bilateral ovaries was removed.

After 2 weeks of recovery, the rats were divided into four groups (each group n = 8) as follows: sham control (sham), ovariectomized model (OVX), ovariectomy + E2 0.27 mg/kg (OVX + E2), and ovariectomized + dehydroepiandrosterone 13.3 mg/kg (OVX + DHEA) groups. The dose of E2 (Abbott, Abbott Park, Ill.,USA) and DHEA (capsule; Puritan's Pride, USA) was calculated based on their doses recommended for women. E2 and DHEA were given daily by gavage for 12 weeks to the rats in the OVX + E2 and OVX + DHEA groups, and the same amount of normal saline was administered to the remaining two groups.

2.3. Measurement of body weight, blood glucose, and lipids

The rats were weighed regularly every Monday at 08:00 h, and the data were recorded once a week. Oral glucose tolerance test (OGTT) was performed using the OneTouch glucose monitor (Johnson & Johnson, Shanghai) at the end of the study. Glucose level in the blood from the tail vein was measured at 0, 30, 60 and 120 min after glucose administration at a dose of 2 g/kg (i.g.) at 08:00 h after fasting overnight. After placing at room temperature for 30 min, the collected blood samples were centrifuged at 3000 rpm for 10 min, and the supernatant was used for further analyses. Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) levels were determined using an automatic biochemical analyzer [24].

2.4. Measurement of depression-like behaviors

2.4.1. Sucrose preference test (SPT)

Each rat was housed in a single cage. Two bottles containing 1% sucrose water were placed on each side of the cage, and the bottles were changed after 12 h. After 24 h, the bottles were replaced with a bottle of pure water and a bottle of 1% sucrose water on the left and right sides of the cage, respectively. The bottles were switched every 12 h. The two water bottles were weighed before and after 24 h to calculate the percentage of sugar water consumption from total water consumption.

2.4.2. Open field test (OFT)

The OFT was performed as previously described in rodents [24, 25]. Briefly, the rats were individually placed in a white plexiglass box (160 cm × 160 cm × 25 cm); the bottom of the box was divided into 16 identical squares in a dimly lit room. The frequency of line crossings (four paws placed in the new box) and rearing numbers (two front paws lifted from the floor) were recorded for 5 min after placing the rats at the center of the cage. The behaviors were recorded using a camera located 180 cm above the box.

2.4.3. Forced swimming test (FST)

The FST was performed in a transparent standard cylinder as described previously under the following conditions: height of water, 25 cm and temperature of water, 22°C–25 °C [26]. The rats were allowed to swim in the water 10 min before the test. Thereafter, immobility duration of rats in the pool was recorded for the next 24 h. Immobility duration was defined as the total time that a rat remained stable or presented only a slight limb swing to maintain its balance in the water. The rats were removed and dried after the experiment, and then returned to the cage.

2.5. Western blotting

The rats were sacrificed 24 h after the behavioral test and the hippocampus from each rat was carefully removed and placed on ice. Subsequently, the hippocampus was homogenized, incubated, and centrifuged; then, the supernatant was collected for further analysis. Protein concentration in the supernatant was measured using the Bicinchoninic Acid Protein Assay Kit (Beyotime, Jiangsu, China). Proteins were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% bovine serum albumin for 1 h, and was then incubated with an appropriate primary antibody overnight at 4 °C. Thereafter, the membrane was incubated with a secondary antibody for 1 h. Finally, the membrane was placed in an exposure apparatus for color reaction. The primary antibodies were as follows: BDNF (1:1000), ERK (1:1000), p-ERK (1:1000), CREB (1:1000), and p-CREB (1:1000) from Cell Signaling Technology; ERα (1:1000) from Abcam; and ERβ (1:1000) and GAPDH (1:5000) from Affinity. The grey levels of the ERα, ERβ, and BDNF (mature) bands were normalized to the GAPDH level, and the pERK and pCREB band intensities were normalized to the ERK and CREB intensities, respectively.

2.6. Statistical analysis

The experimental data are presented as mean ± standard error of the mean (SEM). Homogeneity of variance was tested. Homogeneous data were compared among the four groups and between two groups using the analysis of variance (ANOVA) and Bonferroni test, respectively. Non-homogeneous data were compared using Kruskal–Wallis test. Differences with a P value of <0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics 17.0.

3. Results

3.1. Effects of DHEA on body weight and glucose tolerance in ovariectomized rats

The curve of OGTT is presented in Figure 1A, furthermore, the corresponding area under the curve (AUC) is presented in Figure 1B. The
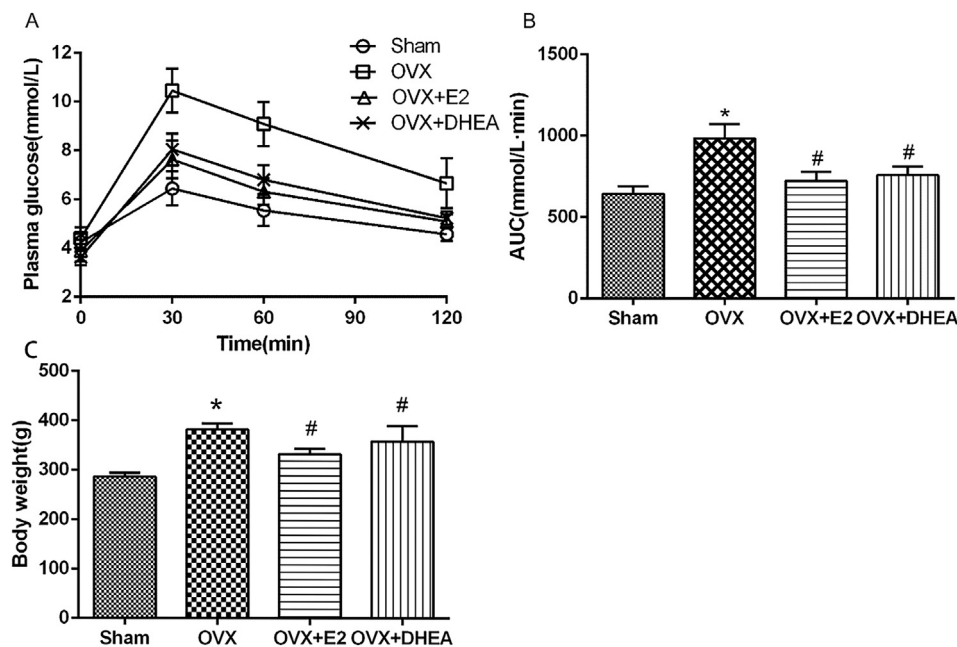
statistics revealed significant difference in the AUC for four groups (F = 34.12, P < 0.001), the multiple comparisons showed that the AUC of the OVX group was significantly increased compared with that of the sham group (P < 0.05), E2 or DHEA treatment for 12 weeks reduced significantly the AUC of OGTT compared to the OVX group (both P < 0.05). As shown in Figure 1C, there is different significantly in the body weight of four groups rats (F = 33.00, P < 0.001). The body weight of the OVX rats was significantly increased at the end of the experiment compared to that of the sham rats (P < 0.05). Furthermore, the DHEA treatment, similar to the E2 treatment, alleviated weight gain caused by ovariectomy after 12 weeks of administration (both P < 0.05).

3.2. Effect of DHEA on blood lipids in ovariectomized rats

The one-way ANOVA statistics showed a significant difference in the TG (F = 5.40, P = 0.004, Figure 2A), TC levels (F = 11.05, P < 0.001, Figure 2B) and LDL-C level (F = 8.40, P = 0.004, Figure 2D), but not difference in HDL-C levels (Figure 2C) among four groups. Bilateral ovarian ablation surgery resulted in a significant increase in the TG (P < 0.05) and TC levels (P < 0.05), and LDL-C level (P < 0.05). While the E2 and DHEA treatments reduced this tendency, but the difference was not significant in the TG and TC levels. Furthermore, the LDL-C level was lower in the E2 treated rats than that in the OVX rats (P < 0.05). However, only a downward trend was presented in DHEA treated group.

3.3. Effects of DHEA on depressive-like behaviors in ovariectomized rats

As shown in Figure 3, there are significant difference in the sucrose preference index (F = 43.22, P < 0.001, Figure 3A), the frequency of line crossing (F = 5.61, P = 0.009, Figure 3B), rearing (F = 5.37, P = 0.01, Figure 3C) and the immobility duration (F = 40.11, P < 0.001, Figure 3D). The sucrose preference index, the frequency of line crossing and rearing was lower, and the immobility duration was longer in the OVX group than those in the sham group (all P < 0.05). Whereas the sucrose preference index in the treatment with E2 and DHEA was higher than that in the OVX group (both P < 0.05). The frequency of line crossing and rearing in the OFT increased significantly in the DHEA or E2 treated group compared to the OVX group, respectively. As shown in
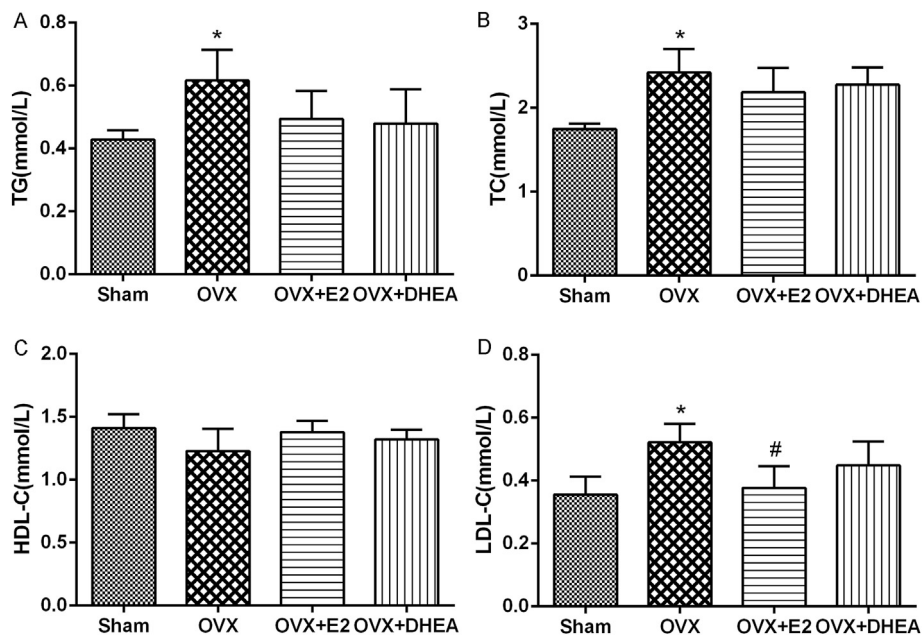

Figure 1. Effects of DHEA treatment on body weight, glucose tolerance in the OVX rats. Oral glucose tolerance test (OGTT) curve (A), the corresponding area under the curve (AUC) (B), and body weight (C) among the four groups. Chronic treatment with DHEA or E2 reduced the AUC of OGTT and body weight gain in the OVX rats. Data were shown as the mean ± SEM (n = 8). *P < 0.05 compared to the sham group; #P < 0.05 compared to the OVX group.

Figure 2. Effects of DHEA treatment on blood lipids in the OVX rats. The figure presented the Level of triglycerides (TG) (A), total cholesterol (TC) (B), HDL-C (C), and LDL-C (D) in rats. The TG, TC, and LDL-C levels were higher in the OVX group than in the sham rats. The LDL-C level was reversed in the E2 group, but not in DHEA treated group. Data were shown as the mean ± SEM (n = 8). *P < 0.05 compared to the sham group; #P < 0.05 compared to the OVX group.
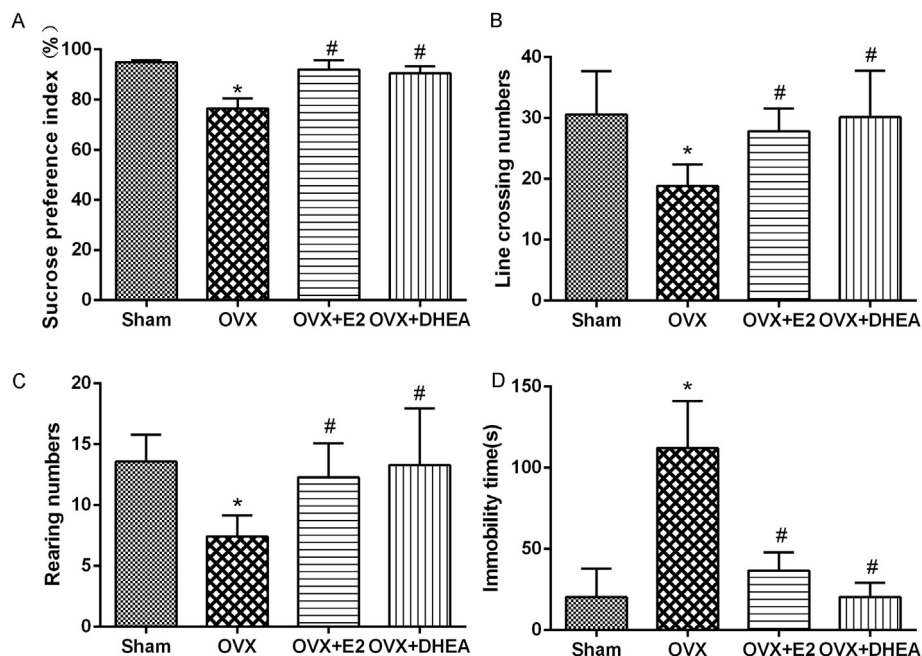

Figure 3. Effects of DHEA treatment on the depressive-like behaviors. Sucrose preference index in the sucrose preference test (A), line crossing numbers (B), and rearing numbers (C) in the open field test; immobility time (D) in the forced swimming test. Long-term supplement of DHEA or E2 could reversed the depressive-like behaviors in the OVX rats. Data were shown as the mean ± SEM (n = 8). *P < 0.05 compared to the sham group; #P < 0.05 compared to the OVX group.

Figure 3D, the duration of immobility in the water apparently reduced after treatment with E2 (P < 0.05) and DHEA (P < 0.05) compared to the OVX group.

3.4. Effects of DHEA on the ERK/CREB/BDNF signaling pathway in the hippocampus of ovariectomized rats

As shown in Figure 4, there was a significant difference in the phosphorylation level of ERK (F = 434.58, P < 0.001, Figure 4A) and CREB (F = 175.72, P < 0.001, Figure 4B) in the hippocampus among the four groups. The expression of both phosphorylated ERK and CREB in the OVX group was lower than that in the sham group, and this tendency was reversed by E2 (P < 0.05) and DHEA supplementation (P < 0.05). Correspondingly, the statistics revealed the significant difference of the expression of BDNF in the hippocampus among the four groups of rats (F = 17.79, P < 0.01), and the expression of BDNF was also upregulated in the E2 (P < 0.05) or DHEA (P < 0.05) treated group compared with that in the OVX group (Figure 4C).
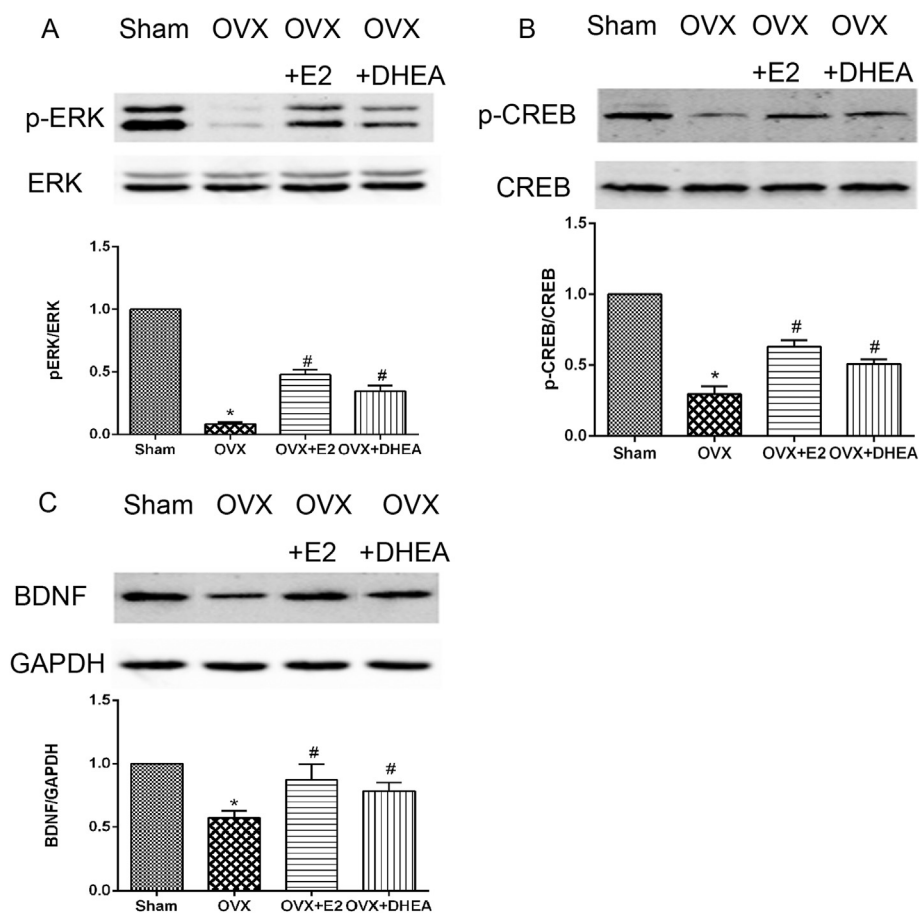
Figure 4. Effects of DHEA on the ERK/CREB/BDNF signaling pathway in the hippocampus. The representative bands and their relative expression histogram of Phosphorylation level of ERK (A), phosphorylation level of CREB (B), and BDNF(C) in the hippocampus are shown. DHEA or E2 treatment increased the expression of p-ERK, p-CREB, and BDNF in the hippocampus. Data were shown as the mean ± SEM (n = 4). *P < 0.05 compared to the sham group; #P < 0.05 compared to the OVX group.

3.5. Effects of DHEA on the expression of ERα and ERβ in the hippocampus of ovariectomized rats

The expression of ERβ in the hippocampus is presented in Figure 5A. The expression of ERβ in the hippocampus was significantly different among the four groups (F = 99.53, P < 0.001). The expression of ERβ was downregulated in the OVX group compared with that in the sham group (P < 0.05). After the administration of E2 (P < 0.05) and DHEA (P < 0.05), the expression of ERβ increased compared with that in the OVX group. There was no significant difference in the expression of ERα in the hippocampus (F = 1.25, P > 0.05, Figure 5B) among the four groups.

4. Discussion

The present findings were that ovariectomy resulted in the glucose and lipid metabolism dysfunction and produce of depressive-like behaviors. Long-term exogenous supplementation of DHEA and E2 improved glucose metabolism and depressive-like behaviors, and partially improved lipid metabolism via the ERK/CREB/BDNF signaling pathway in the hippocampus. ERβ in the hippocampus might be involved in the antidepressant effect of DHEA. Our study provides a strong basis for the use of DHEA as a perimenopausal HRT, especially for perimenopausal or sex hormone deficiency women with depression-like behaviors.

Estrogen and its receptors can act on the hypothalamus to reduce feeding behavior and energy intake by regulating energy-regulating factors [27]. A lack of estrogen in the body promotes fat accumulation and insulin resistance. Furthermore, as adipose tissue increases, the levels of inflammatory substances, low-density lipoproteins, triglycerides and fatty acids, and sensitivity to insulin decrease [28], even resulting in type 2 diabetes. Restoration of E2 to the physiological concentration will help maintain insulin activity and glucose tolerance [29]. It has been

shown that DHEA can inhibit the storage of energy by increasing fatty acid β-oxidation, promoting metabolic heat production, and mitigating triglyceride level, thus contributing to weight loss [30]. Furthermore, DHEA can decrease glucose synthesis by inhibiting glucose-6-phosphatase and restrain dexamethasone-induced renal gluconeogenesis [31]. In the present study, while the administration of DHEA improved glucose tolerance, it may increase insulin sensitivity. The level of TG, TC, and LDL-C levels increased in the OVX rats, supplement of DHEA also has some improvements of blood lipids. Thus, our data demonstrated that DHEA may improve body weight, glucose metabolism, and lipid metabolism to some extent in the menopausal stage or in hypoestrogenic state.

Estrogen has neuroprotective effects and regulates emotional events by promoting the formation of hippocampal synaptic plasticity [32, 33, 34]. Frequent fluctuations in the estrogen level in perimenopausal a or postpartum women can lead to the development of depressive symptoms in them [35]. In rodents, ovariectomy is commonly used to establish perimenopausal syndrome in rats [36]. After bilateral ovarian ablation, the estrogen level in rats reduced, which could cause a series of mood disorders. Meanwhile, ovariectomy can impair spatial memory, synapse generation, and long-term potentiation, and estrogen supplementation can reverse these changes [37]. The sucrose preference is designed to measure anhedonia, one of the major symptoms of depression [38]. The OFT is used to assess the ability of action and the behavior to explore [39]. The FST reflects the desperation of rats in water and their desire to survive under irresistible pressure. In the present study, the OVX rats showed less interest in nature rewards, and they were inactive with a lower frequency of line crossings and longer duration of immobility in the water. Furthermore, the OVX rats lacked curiosity and rarely stood in the OFT. Additionally, they were more desperate in the water and easily gave up. These behaviors were reversed by DHEA supplementation for 12
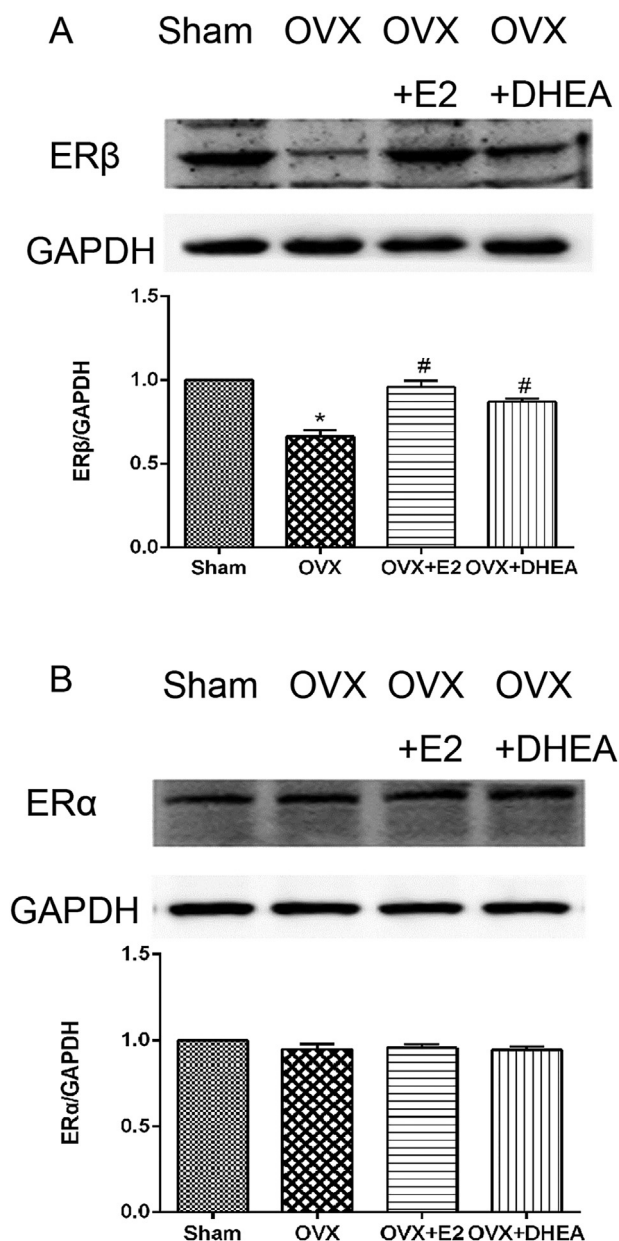
Figure 5. Effects of DHEA on the expression of ERβ and ERα in the hippocampus. The representative bands and their relative expression histogram of ERβ (A) and ERα (B) in hippocampus are shown. After 12 weeks of E2 and DHEA administration, the expression of ERβ was upregulated significantly. Data were shown as the mean ± SEM (n = 4). *P < 0.05 compared to the sham group; #P < 0.05 compared to the OVX group.

weeks. DHEA produces a variety of steroid hormones, including androgens and estrogens, and it is closely related to the pathogenesis of depression [40]. Thus, DHEA-induced behavioral changes observed in the present study, similar to E2, can ameliorate depression-like behaviors caused by estrogen deficiency.

BDNF is highly expressed in the hippocampus, amygdale, and frontal cortex, and it plays an important role in the differentiation and formation of synapse and regeneration of various neurons. BDNF is also involved in the pathogenesis of depression [41]. When neurons are degenerated or damaged, BDNF can exert protective effects by increasing the survival time of neurons and promoting neuronal repair [42]. Preclinical data have shown that depressive symptoms are always accompanied by a decrease in the BNDF level and that antidepressant treatment can increase it [23]. Our results also confirmed that after ovarian ablation, the

level of BDNF in the hippocampus was significantly reduced. DHEA supplementation increased the expression of BDNF in the hippocampus. Furthermore, the phosphorylation of ERK and CREB was significantly decreased in the hippocampus of OVX rats, and this was reversed by DHEA supplementation. The improvement in depression-like behaviors by DHEA was consistent with the increased expression of BDNF and phosphorylation of ERK and CREB in the hippocampus. Thus, the antidepressant effect of DHEA might involve the activation of BDNF and the ERK/CREB signaling pathway in the hippocampus.

Estrogen regulates the expression of BDNF via the estrogen response element in the gene encoding BDNF [43]. In the present study, we evaluated the expression of the two subtypes of estrogen receptors, namely ERα and ERβ, in the hippocampus. The expression of ERβ, rather than ERα, was downregulated in the hippocampus of the OVX rats. The supplementation of DHEA and E2 upregulated the expression of ERβ, indicating that ERβ expression may be involved in the antidepressant effect of DHEA. These results are consistent with those of a previous study, which reported that ERβ is involved in the antidepressant effect of estrogen [44]. It has also been reported that the antidepressant effect of estrogen can be reversed in ERβ knockout mice [45]. Moreover, the evidence presents that ERβ knockout female mice shows reduced BDNF expression in hippocampus but not in the ERα knockout mice [46], the present data may buttress this by suggesting that DHEA may achieve its positive effects in this model by modulation ERβ expression, in addition of activating the receptor. Thus, DHEA and E2 may upregulate ERβ expression, subsequently activate the expression of BDNF in the hippocampus to improve depression-like behaviors.

Furthermore, DHEA replacement therapy may not increase the incidence of endometrial cancer. Studies have shown that oral DHEA does not adversely affect endometrial menopausal women, and this may be related to the lack of aromatase in the endometrium required for the conversion of DHEA to estrogen [16]. Cautiously, the risk of ovarian cancer may increase with higher DHEAS levels in blood [14], and supplementation of exogenous DHEA may increase the risk of breast cancer in postmenopausal women with obesity [15]. Whereas, DHEA has been reported to inhibit breast cancer development by inhibiting the proliferation and migration of breast cancer cells, probably due to the shortage of enzymes required for the conversion of DHEA to estrogen [47]. Overall, DHEA may be adopted as a treatment for perimenopausal depression or PPD, especially in patients with abnormal glucose and lipid metabolism; however, further clinical trials are needed to further validate our results.

5. Conclusions

Here, we demonstrated that DHEA, similar to E2, improves depressive-like behaviors in OVX rats, ameliorates perimenopausal obesity, and reverses glucose tolerance. DHEA increased the expression of BDNF by activating the ERK/CREB signaling pathway in the hippocampus. ERβ might be an important target in this process. The results suggest that DHEA can be used as an adjuvant to treat perimenopausal or sex hormone deficiency women with depression.

Declarations

Author contribution statement

S. Wu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.Ye: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Z. Li: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

S. Bu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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