

Vitamin D and 17 β -estradiol upregulate each other's receptors and regulating the AMPK/NF- κ B pathway to relieve depressive-like behaviors in female ovariectomized rats

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Research

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Abstract

Background: A deficiency of vitamin D (VD) or 17 β -estradiol (E2) is associated with increased risk of mood disorders such as depression in menopausal females, but the mechanism underlying is still elusive. The present study aims to evaluate whether vitamin D and 17 β -estradiol could relieve a depressive-like state through neuroinflammatory regulation in ovariectomized (OVX) rats.

Methods: Female SD rats were randomly divided into four groups, namely, control (SHAM), OVX, OVX+VD, and OVX+E2. The treatment procedure was performed for 10 weeks until sacrifice.

Results: The chronic administration of vitamin D and 17 β -estradiol showed anti-depressive-like activity in the OVX rats. Additionally, vitamin D and 17 β -estradiol upregulated each other's receptors, including VDR, ER α , and ER β in the hippocampus of OVX rats. Vitamin D and 17 β -estradiol showed neuroprotective effects by decreasing OVX-induced apoptosis and neuronal damage, regulating the AMPK/NF- κ B signaling pathway, and reducing the proinflammatory cytokines (IL-1 β , IL-6, and TNF α), as well as iNOS and COX-2 in the hippocampus of OVX rats.

Conclusions: The present study demonstrated that vitamin D and 17 β -estradiol could upregulate each other's receptors and regulate the AMPK/NF- κ B pathway to relieve the OVX-induced depressive-like state. The results should stimulate translational research towards the vitamin D potential for prevention or treatment of menopause-related depression.

1. Introduction

The prevalence of depression is dramatically increased in menopausal women [1]. Menopausal disorders, including depression, are strictly related to the loss of ovarian function and a chronic hypo-estrogenic state. One strategy to relieve menopausal disorders is to supply estrogens. However, estrogens increase the risk of endometrial hyperplasia or cancer, which hinders their clinical use [2]. Therefore, it becomes urgent to seek an alternative treatment strategy to relieve menopause-related depression. Vitamin D (VD) supplementation seems like a promising strategy. Vitamin D intake is considered to be safe and is recommended to prevent bone loss in menopausal women. One study found that healthy postmenopausal older women supplemented with vitamin D₃ and calcium did not result in a significant risk change of all-type cancer [3]. Furthermore, low vitamin D level is noted in depressive patients, and vitamin D supplementation has shown beneficial in improving mood [4, 5]. Although vitamin D and 17 β -estradiol (E2) is likely to be improving mood in menopausal women with depressive disorder, the mechanisms underlying remain equivocal.

Neuroinflammation is one of the most essential contributors to depression. Patients with depression are more likely to have a higher status of proinflammatory cytokines in the periphery, cerebrospinal fluid, and hippocampus [6]. Animal studies find that proinflammatory cytokines could induce depressive-like behaviors, and depressive-like models have elevated levels of proinflammatory cytokines [7]. Ovariectomized (OVX) rodent is a widely used menopausal model [8]. Long-term after OVX, animals not

only developed depressive-like behaviors but also showed higher neuroinflammatory levels [9]. Furthermore, estrogen supplementation, exercise, and inflammasome inhibition in the OVX rodents could exert an anti-depressive-like effect by immune-regulating [9, 10], strongly suggesting an important role of the immune system in the OVX-induced depressive-like behaviors.

Nuclear factor-kappa B (NF- κ B) is a protein complex that plays a crucial role in inflammatory regulation, and NF- κ B could be negatively controlled by AMP-activated protein kinase (AMPK) [11]. Under physiological condition, NF- κ B bounding with I κ B maintains in an inactive state in the cytoplasm. Once activated, NF- κ B's inhibitor I κ B is phosphorylated and degraded, resulting in p65 phosphorylation and transcriptional regulation of proinflammatory cytokines and neurotoxic mediators, such as interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) [12][12]. These proinflammatory cytokines and neurotoxic mediators have been proven to involve in the pathophysiology of depression [13]. Therefore, the present study aims to evaluate whether vitamin D and 17 β -estradiol relieve a depressive-like state through neuroinflammatory regulation in the OVX rats.

2. Materials And Methods

2.1 Animals

Experiments were conducted on female Sprague-Dawley (SD) rats (12-week old). All rats were housed under standard laboratory conditions of temperature (23 \pm 2 $^{\circ}$ C), light (12h light/dark cycle), and a relative humidity (55 \pm 10%), with free access to food and water. The rats were habituated for 7 days before the experiment. All animal use procedures were conducted by the ethical standards in the 1964 Declaration of Helsinki and its later amendments, with the approval of the Ethics Committee of the Zhongshan Affiliated Hospital of Zhongshan University.

2.2 Drug supplementation and ovariectomy

The rats were randomly assigned into the following four groups (7–8 rats in each group): the sham-operated control (SHAM), the OVX, the OVX+VD, and the OVX+E2 group. The bodyweight of the rats was measured in the experiment. In the OVX group, after full anesthesia with sodium pentobarbital (50 mg/kg, i.p.), the ovaries, oviducts, and top of the fallopian tubes were bilaterally removed through two small incisions. In the SHAM group, similar protocols were conducted without ovariectomy. Since the day of OVX surgery, vitamin D (calcitriol, 100 ng/kg, Roche, China) and 17 β -estradiol (30 μ g/kg, Macklin, Shanghai, China) had been daily gavaged in the OVX+VD and OVX+E2 group, respectively. Calcitriol at a dose of 100ng/kg has been proven to enhance the VDR protein level without affecting serum calcium and phosphate status in our previous study [14]. The treatment procedure was performed for 10 weeks until sacrifice. The animals in the SHAM group were sacrificed at the diestrus phase to avoid the effects of the estrus cycle. Ten weeks later, the forced swim test (FST) and novelty-suppressed feeding test (NSFT)

were carried out. Twenty-four hours after the behavioral tests, the rats were anesthetized with a peritoneal injection of sodium pentobarbital (50mg/kg). Serum was separated by centrifuging at 4000 rpm for 10min at 4 °C. The uterine were collected and weighted. Hippocampus samples were thoroughly washed with cold physiological saline. All the samples were then stored at -80 °C until analysis.

2.3 Behavioral tests

The FST was conducted according to a classic paradigm with minor modifications to measure behavioral despair in stressful situations [15]. The FST contained two trials. In the first trial, the rats were individually forced to swim for 15 min in a Plexiglas cylinder (45cm height, 25cm diameter) containing approximately 35cm of water (24±1°C). The rats were dried and removed to their home cages. In the second trial, the rats were placed again in the cylinders for 5 min 24 h later, and this test session was videotaped. Immobility is defined as floating motionless or making slight movements to keep its head just above water. The duration of immobility was measured by an experienced observer who was blinded to the experimental design.

The NSFT was adapted from a previous study [16]. Briefly, the rats were deprived food for 24 h before the test. The rats were then placed in an open field (75×75×40 cm), where the rats were free to explore for 8min. There is a small amount of food placed on a piece of white paper (10×10 cm) in the center of the field. The latency to feed is defined as the duration between placing the rats in the open field and they take the first bite of the food. After the test, the rats were immediately sent back to their home cage, and the total food consumption for the next 5 min was weighed to avoid the influence of the rats' appetite.

2.4 Hormone assay

Concentrations of 17β-estradiol in the serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Guxi Biotech, China) following the manufacturer's protocol.

2.5 Western Blot Analysis

Total hippocampal protein was prepared, and the concentrations were determined using the Bradford method. The protein samples were loaded into a precast 12% SDS-PAGE gels with approximately 50 µg of protein in each lane. Subsequently, the proteins in the gels were transferred to a PVDF membrane and blocked for 1 hour in 5% nonfat dry milk in TBS-T (150mM NaCl, 25mM Tris, pH 7.5, 0.05% Tween-20). The antibodies and concentrations listed below were used overnight at 4 °C: vitamin D receptor (VDR) (Santa Cruz, 1:300), estrogen receptor α (ERα) (Proteintech; 1:1000), estrogen receptor β (ERβ) (Proteintech; 1:3000), cleaved caspase3 (Cell Signaling; 1:1000), phosphor-AMPK (Thr172) (Cell Signaling; 1:2,000), AMPK (Cell Signaling; 1:2,000), NF-κB inhibitor (IκB, Abcam, 1:1000) NF-κB (Cell Signaling, 1:1000), and β-actin (Proteintech; 1:4000). The membrane was then probed with an HRP-

conjugated secondary antibody for 40min. The film signals were digitally scanned and quantified using Image J software. The signals were normalized to β -actin as an internal standard.

2.6 Quantitative real-time PCR

Total RNA was isolated from the hippocampal homogenates using Trizol reagent (Invitrogen, USA). The mRNA expression of calbindin-d28k (cabp-d28k), calbindin-d9k (cabp-d9k), IL-1 β , IL-6, TNF- α , iNOS, and Cox2 was detected. Their oligonucleotide primers specific for rats are listed in table 1. Quantitative RT-PCR was performed on a Bio-Rad Cx96 Detection System (Bio-Rad, USA) using a SYBR green PCR kit (Applied Bio-systems, USA). The 5 ng cDNA samples received 40 cycles of amplification. Each cDNA was examined in triplicate. Relative quantitation for PCR product was normalized to β -actin as an internal standard.

2.7 Apoptosis Analysis

Apoptosis examination was carried out using a commercially kit (Keygen Biotech, Nanjing, China). The hippocampal samples were fixed in 10% phosphate-buffered paraformaldehyde, embedded in paraffin, and then cut into 6 mm thick sections. The apoptosis in the hippocampal CA1 region was detected using the TUNEL method, which checks the fragmentation of DNA in the nucleus during apoptotic cell death *in situ*.

2.8 Nissl's staining

Nissl's staining could detect the Nissl body in cytoplasm and dendrites of neurons, and this stain has been widely used to identify neuronal damage. The Nissl's staining was carried out according to a conventional method [17]. The hippocampal CA1 region was carefully observed in three randomly selected fields of view by an optical microscope (Leica DFC420, Germany) at a magnification of 200 \times . Image J software was used to count the stained cells. According to a previous method [18], the average number of viable neurons per mm² was calculated.

2.9 Immunofluorescent staining

Paraffin-embedded sections of the hippocampus were dewaxed in xylol, rehydrated, and rinsed in PBS. Antigen retrieval was performed by boiling the sections in a citric acid buffer (0.01 mol/L, pH 6.0), followed by blocking with 5% goat serum for 1h at room temperature. Subsequently, the sections were incubated with the primary antibody, anti-ionized calcium-binding adapter molecular 1 (Iba-1) (Abcam, 1:200) or VDR (Santa Cruz, 1:200). The sections were then washed with PBS three times and stained with 4',6-diamidino-2-phenylindole (DAPI) (Beyotime Biotechnology, China) to visualize cell nuclei. Immunofluorescent images were taken with an inverted fluorescence microscope (Olympus, Japan), and

images were then analyzed using the Image J software to obtain the mean fluorescence density of each visual field in the hippocampal CA1 region.

2.10 Statistical Analysis

Statistical procedures were conducted in SPSS version 18 software. Data were shown as the means \pm SD. Differences between groups were determined by one-way ANOVA. A prior level of significance was established at $P < 0.05$. Post hoc comparisons were made using the Tukey test.

3. Results

3.1 Effects of vitamin D and 17 β -estradiol supplementation on the bodyweight, uterine weight, estradiol level, and depressive-like behaviors

OVX markedly decreased the uterine weight ($P < 0.01$, Fig. 1c), decreased serum 17 β -estradiol level ($P < 0.01$, Fig. 1d), and increased the bodyweight growth ($P < 0.01$, Fig. 1b) at the end of experiment in comparison to the SHAM group, indicating that the animal model was successfully built. Vitamin D treatment had no significant effect on the 17 β -estradiol level ($P > 0.05$, Fig. 1d), bodyweight ($P > 0.05$, Fig. 1b), and uterine weight ($P > 0.05$, Fig. 1c) in comparison to the OVX group. However, 17 β -estradiol treatment restored the 17 β -estradiol level in the serum ($P < 0.01$, Fig. 1d), decreased the bodyweight ($P < 0.01$, Fig. 1b), and increased uterine weight ($P < 0.01$, Fig. 1c) in comparison to the OVX group. Nonetheless, both vitamin D and 17 β -estradiol alleviated the OVX-induced depressive-like behaviors with decreased immobility time ($P < 0.05$ and $P < 0.01$, respectively, Fig. 1e) in the FST and decreased latency time ($P < 0.01$, Fig. 1f) in the NSFT in the OVX rats.

3.2 Vitamin D and 17 β -estradiol enhanced the expression of each other's receptors in the hippocampus of OVX rats

Chronic OVX decreased the expression of VDR ($P < 0.01$, Fig. 2b), cabp-d28k ($P < 0.01$, Fig. 2c), cabp-d9k ($P < 0.01$, Fig. 2d), and ER β ($P < 0.01$, Fig. 2g), but ER α ($P > 0.05$, Fig. 2f) was not influenced in comparison to the SHAM group. Interestingly, vitamin D and 17 β -estradiol supplementation both enhanced the expression of VDR ($P < 0.01$, Fig. 2b), cabp-d28k ($P < 0.01$, Fig. 2c), cabp-d9k ($P < 0.01$ and $P < 0.05$, respectively, Fig. 2d), ER α ($P < 0.05$ and $P < 0.01$, respectively, Fig. 2f), and ER β ($P < 0.01$, Fig. 2g) in comparison to the OVX group. The results of immunofluorescent staining could further visually support that vitamin D and 17 β -estradiol both enhanced the VDR expression (Fig. 2a) in the OVX rats.

3.2 Neuroprotective effects of vitamin D and 17 β -estradiol in the hippocampus of OVX rats

Chronic OVX induced the abundance of Tunel-positive cells (Fig. 3a), increased apoptotic index ($P < 0.01$, Fig. 3c), increased cleaved caspase3 ($P < 0.01$, Fig. 3e), and decreased the number of viable neurons ($P < 0.01$, Fig. 3d) in comparison to the SHAM group, indicating that the hippocampal apoptosis and neuronal damage were significantly increased in the OVX rats. Vitamin D and 17 β -estradiol both showed neuroprotective effects by decreasing Tunel-positive cells (Fig. 3a), decreasing apoptotic index ($P < 0.01$, Fig. 3c), decreasing cleaved caspase3 ($P < 0.05$ and $P < 0.01$, respectively, Fig. 3e), and increasing the number of viable neurons ($P < 0.01$, Fig. 3d) in the OVX rats.

3.3 Effects of vitamin D and 17 β -estradiol on the AMPK/NF- κ B signaling pathway in the hippocampus of OVX rats

To further confirm the immune-regulatory effect of vitamin D and 17 β -estradiol, the AMPK/NF- κ B signaling pathway was assessed. The results showed that OVX decreased the p-AMPK/AMPK ratio ($P < 0.01$, Fig. 4a), decreased the I κ B protein expression ($P < 0.01$, Fig. 4b), and increased the NF- κ B protein level ($P < 0.01$, Fig. 4c) in comparison to the SHAM group. The supplementation of vitamin D and 17 β -estradiol enhanced the p-AMPK/AMPK ratio ($P < 0.01$, Fig. 4a), suppressed the NF- κ B protein level ($P < 0.01$, Fig. 4c), and increased the I κ B protein expression ($P < 0.05$ and $P < 0.01$, respectively, Fig. 4b) in the OVX group.

3.4 Effects of vitamin D and 17 β -estradiol on proinflammatory cytokines, iNOS, and COX-2 in the hippocampus of OVX rats

There was a remarkable increase of IL-1 β ($P < 0.01$, Fig. 5c), IL-6 ($P < 0.01$, Fig. 5d), TNF α ($P < 0.01$, Fig. 5e), iNOS ($P < 0.01$, Fig. 5f), and COX-2 ($P < 0.01$, Fig. 5g) in the OVX rats in comparison to the SHAM rats. Vitamin D and 17 β -estradiol significantly decreased the gene expression of IL-1 β ($P < 0.01$, Fig. 5c), IL-6 ($P < 0.01$, Fig. 5d), TNF α ($P < 0.01$, Fig. 5e), iNOS ($P < 0.05$ and $P < 0.01$, respectively, Fig. 5f), and COX-2 ($P < 0.01$, Fig. 5g) in the OVX rats. Immunofluorescence assays found that vitamin D and 17 β -estradiol significantly decreased the OVX-induced over-expression of Iba-1 positive cells ($P < 0.01$, Fig. 5b) in the hippocampal CA1 region.

4. Discussion

In this study, the chronic administration of vitamin D and 17 β -estradiol showed anti-depressive-like activity in the OVX rats. Additionally, we provide evidence that vitamin D and 17 β -estradiol could

upregulate each other's receptors, including VDR, ER α , and ER β in the hippocampus of OVX rats. Vitamin D and 17 β -estradiol showed neuroprotective effects by decreasing OVX-induced apoptosis and neuronal damage, regulating the AMPK/NF- κ B signaling pathway, and decreasing proinflammatory cytokines (IL-1 β , IL-6, and TNF α), as well as iNOS and COX-2 in the hippocampus of OVX rats. These changes might contribute to, at least in part, the anti-depressive-like activity of vitamin D and 17 β -estradiol.

We found that chronic OVX induced depressive-like behaviors with decreased immobility time in the FST and decreased latency time in the NSFT, in line with a previous study that OVX developed depressive-like behaviors at more than 6 weeks [19]. The OVX model was successfully built, as evidenced by the increased bodyweight, decreased uterine weight, and decreased estrogen level. These basic characteristics were recovered by 17 β -estradiol but not vitamin D, although they both showed an anti-depressive-like effect. The results indicate that the anti-depressive-like effect of vitamin D is estrogen-independent.

The most striking finding in this study is that vitamin D and 17 β -estradiol could upregulate each other's receptors in the hippocampus of OVX rats, indicating a crosstalk between vitamin D and 17 β -estradiol in relieving depressive-like behaviors. A similar finding was reported in marrow stromal cells that the treatment of vitamin D and 17 β -estradiol upregulated each other's receptors (VDR and ER α) [20]. Vitamin D has numerous biological functions and acts through its receptor (VDR) in most cells. The co-elevated expression of VDR and calcium-binding protein (cabp-d28k and cabp-d9k) suggests that the vitamin D signaling system was amplified by vitamin D and 17 β -estradiol in the hippocampus of OVX rats. ERs are essential for memory consolidation [21]. In this study, we found that OVX caused an abrupt decrease of ER β but not ER α . A similar finding was reported in a depressive rodent model that chronic unpredictable mild stress decreased ER β protein level but not ER α in the hippocampus of mice [22]. Furthermore, OVX-induced depressive-like behaviors could be reversed by administration of 17 β -estradiol and ER β agonist but not ER α agonist in mice [9]. The results together indicate that the decreased ER β level might be responsible for depressive-like behaviors in the rodents.

In morphology, OVX caused significantly neuronal apoptosis, damage, and neuroimmune overactivation, as evidenced by the fact that the Tunel-positive cells remarkably increased, viable neurons declined, and Iba-1 immunofluorescent enhanced, respectively. Vitamin D and 17 β -estradiol ameliorated the OVX-induced neuronal apoptosis, damage, and neuroimmune overactivation in the OVX+VD and OVX+E2 group compared to the OVX group. The results provide visual evidence that vitamin D and 17 β -estradiol have neuroprotective effects.

Neuroinflammation affects every pathological aspects of depression, including neuronal apoptosis, neurogenesis, neurotransmission, and neuroplasticity, playing a key role in the onset and development of depression [23]. Hippocampal inflammation has been widely reported in patients with depression and animal models of depression [24]. NF- κ B serves as a dominated factor in regulating inflammation [25]. We found that the NF- κ B was activated in the OVX group compared to the SHAM group. As expected, vitamin D and 17 β -estradiol effectively re-balanced the AMPK/NF- κ B signaling pathway. The results

highlight that vitamin D and 17 β -estradiol might exert an anti-inflammatory effect through the AMPK/NF- κ B signaling pathway at the state of chronic hypoestrogenism.

Our study correlates with the results from previous literature that long-term OVX could induce a neuroimmune-activated state with increased expression of proinflammatory cytokines [26]. Furthermore, the current study demonstrates that Vitamin D and 17 β -estradiol decreased the gene levels of proinflammatory cytokines (IL-1 β , IL-6, and TNF α) in the hippocampus of OVX SD rats. Another study found that vitamin D only partially reversed the increase of IL-6 protein level in the hippocampus of OVX Wistar rats [27]. Although IL-1 β , IL-6, and TNF α are both proinflammatory cytokines, they might respond diversely in depression. A meta-analysis found profoundly higher levels of TNF- α and IL-6 in the serum of patients with depression but not IL-1 β [28]. Intriguingly, another meta-analysis reported that anti-depressants decreased IL-1 β level and possibly IL-6 but not TNF α [29].

In addition to the proinflammatory cytokines, iNOS and COX-2, which are both the downstream targets of the AMPK/NF- κ B pathway, are also involved in the pathophysiology of depression [13]. iNOS is responsible for the nitric oxide (NO) formation in response to inflammatory mediators, and the inhibition of iNOS showed a potential anti-depressive-like effect in depressive models [30]. COX-2 is a key enzyme for the production of a series of inflammatory cytokines, and the inhibition of COX-2 by meloxicam could relieve depressive-like behaviors [31]. In this study, we found that vitamin D and 17 β -estradiol significantly decreased the OVX-induced overexpression of iNOS and COX-2, which further support the neuroimmune-regulatory effect of vitamin D and 17 β -estradiol.

5. Conclusions

Collectively, the present study demonstrates that vitamin D and 17 β -estradiol exert an anti-depressive-like effect by regulating the AMPK/NF- κ B pathway, and decreasing proinflammatory cytokines (IL-1 β , IL-6, and TNF α), as well as iNOS and COX-2 in the hippocampus of OVX rats. Additionally, vitamin D and 17 β -estradiol could upregulate each other's receptors in the hippocampus of OVX rats, indicating a crosstalk between vitamin D and 17 β -estradiol in relieving depressive-like behaviors. These findings are supporting the evidence that vitamin D and 17 β -estradiol might exert an anti-depressive-like function through neuroinflammatory regulation. Furthermore, these findings should stimulate future translational research towards the vitamin D potential for prevention or treatment of menopause-related depression.

Abbreviations

VD: Vitamin D; E2: 17 β -estradiol; OVX: Ovariectomized; NF- κ B: Nuclear factor-kappa B; AMPK: AMP-activated protein kinase; IL: Interleukin; TNF: Tumor necrosis factor; iNOS: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2; SD: Sprague-Dawley; FST: Forced swim test; NSFT: Novelty-suppressed feeding test; ELISA: Enzyme-linked immunosorbent assay; VDR: Vitamin D receptor; ER: Estrogen receptor; Cabp: Calbindin

Declarations

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Not applicable

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

PJ designed the study and wrote the protocol. Authors WZ, PJ, YG, and KW performed the experiments and analyzed the data. Author WZ and PJ managed the literature searches and figure drawing. WZ wrote the manuscript. All authors contributed to have approved the final manuscript.

Ethics approval

All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee of the Zhongshan Affiliated Hospital of Zhongshan University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1 Primer sequences used for the qPCR analysis

Gene	Sense primer (5'-3')	Antisense primer (5'-3')
cabp-d28k	TTGTGGATCAATATGGGCAGA	ACTTCAGTTGCTGGCATCGA
cabp-d9k	GACCTCACCTGTTCTGTCTG	GCTCCTTCTTCTGGCTTCATT
IL-1 β	AGGTCGTCATCATCCCACGAG	GCTGTGGCAGCTACCTATGTCTTG
IL-6	CACAAGTCCGGAGAGGAGAC	ACAGTGCATCATCGCTGTTC
TNF- α	GAGAGATTGGCTGCTGGAAC	GAGAGATTGGCTGCTGGAAC
iNOS	AGTGGCAACATCAGGTCGG	CGATGCACAACCTGGGTGAAC
COX-2	GCATTCTTTGCCAGCACTT	GTCTTTGACTGTGGGAGGAT
β -Actin	CATCCTGCGTCTGGACCTGG	TAATGTCACGCACGATTTCC

Figures

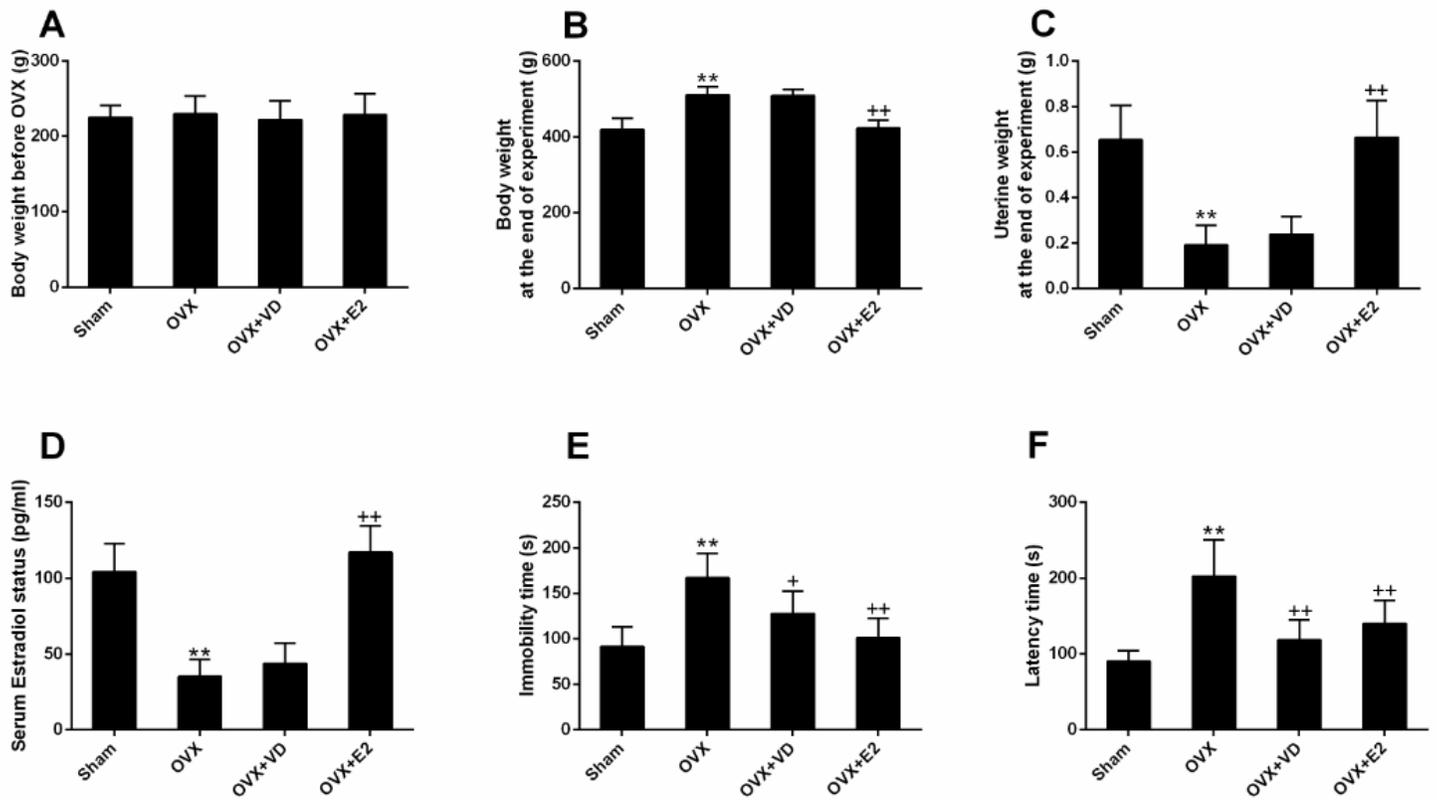


Figure 1

Effects of vitamin D and 17β-estradiol on basic characteristics and depressive-like behaviors in the OVX rats. Statistical graphs of bodyweight before OVX (A), bodyweight at the end of experiment (B), uterine weight at the end of experiment (C), serum 17β-estradiol level (D), immobility time in forced swim test (E), and latency time in novelty-suppressed feeding test (F). Data are the means ± SD (n=7-8). ** P < 0.01 compared to the SHAM group. + P < 0.05, ++ P < 0.01 compared to the OVX group.

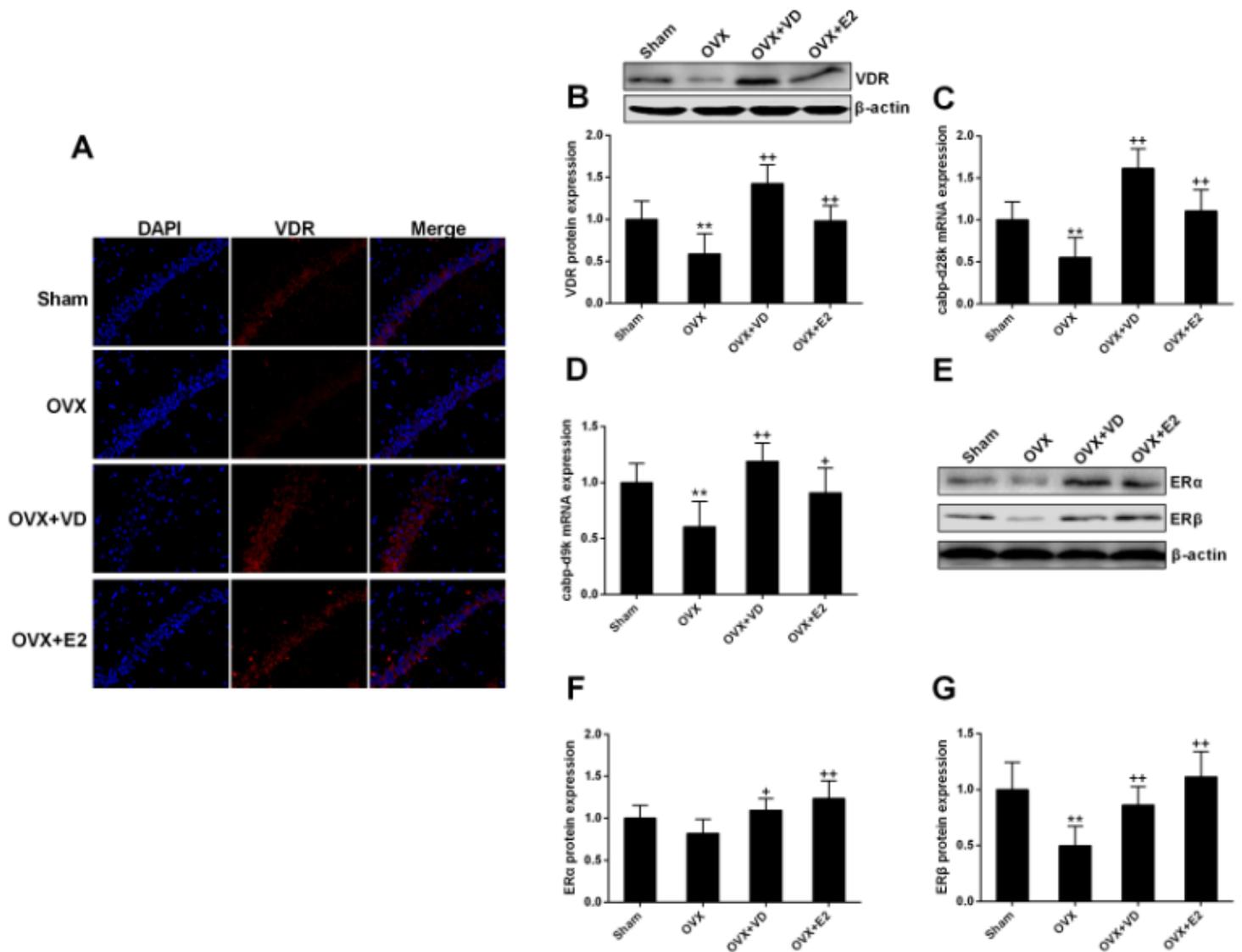


Figure 2

Vitamin D and 17 β -estradiol enhance the expression of each other's receptors in the hippocampus of OVX rats. Representative images of immunofluorescence assays of VDR in the hippocampal CA1 region (A). Representative blots and statistical graphs of VDR (B). Relative mRNA expression of cabp-d28k (C) and cabp-d9k (D). Representative blots (E) and statistical graphs of ER α (F) and ER β (G). Data are the means \pm SD (n=7-8). ** P < 0.01 compared to the SHAM group. + P < 0.05, ++ P < 0.01 compared to the OVX group.

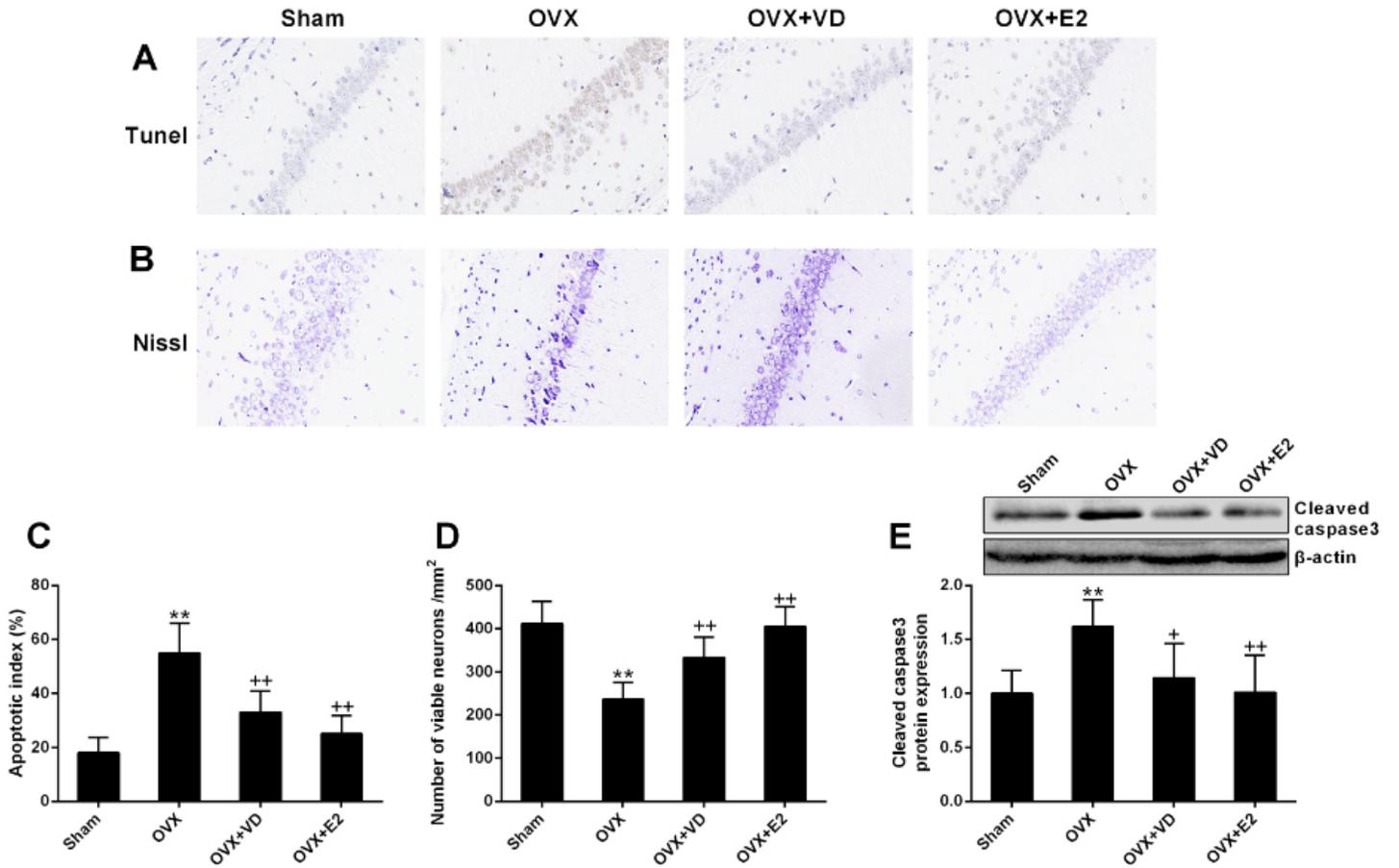


Figure 3

Vitamin D and 17 β -estradiol both show a neuroprotective effect in the hippocampus of OVX rats. Representative images of TUNEL staining (A) and Nissl staining (B) in the hippocampal CA1 region. Statistical graphs of the apoptotic index (C) and the number of viable neurons (D). Representative blots and statistical graphs of cleaved caspase3 (E). Data are the means \pm SD (n=7-8). ** P < 0.01 compared to the SHAM group. + P < 0.05, ++ P < 0.01 compared to the OVX group.

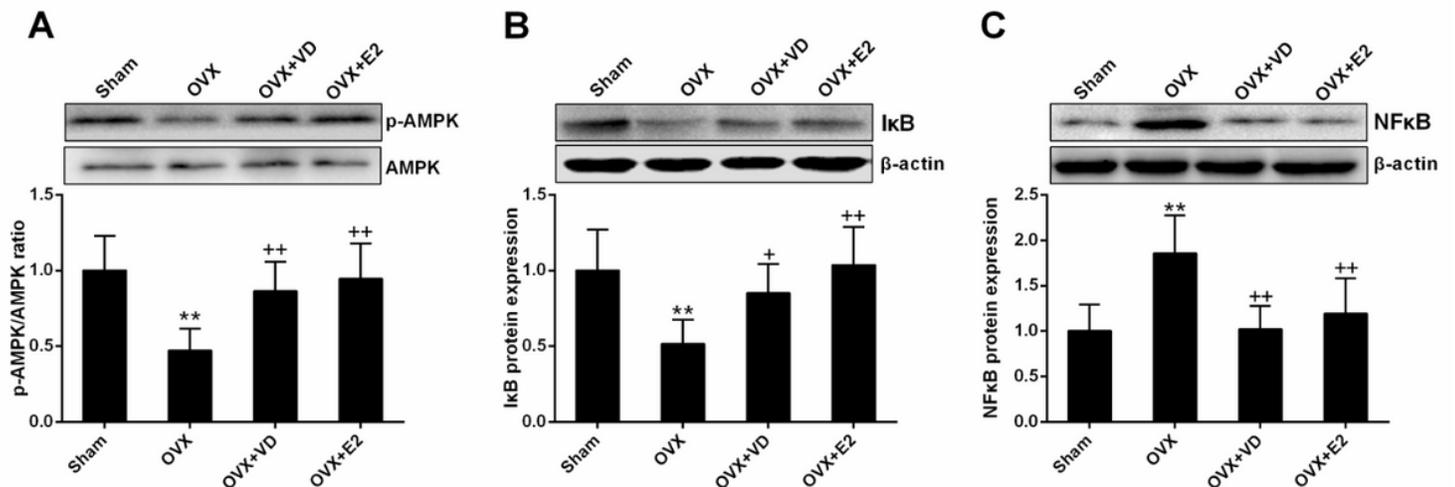


Figure 4

Effects of vitamin D and 17 β -estradiol on the AMPK/NF- κ B pathway in the hippocampus of OVX rats. Representative blots and statistical graphs of p-AMPK/AMPK ratio (A), I κ B (B) and NF- κ B (C). Data are the means \pm SD (n=7-8). * P < 0.05, ** P < 0.01 compared to the SHAM group. ++ P < 0.01 compared to the OVX group.

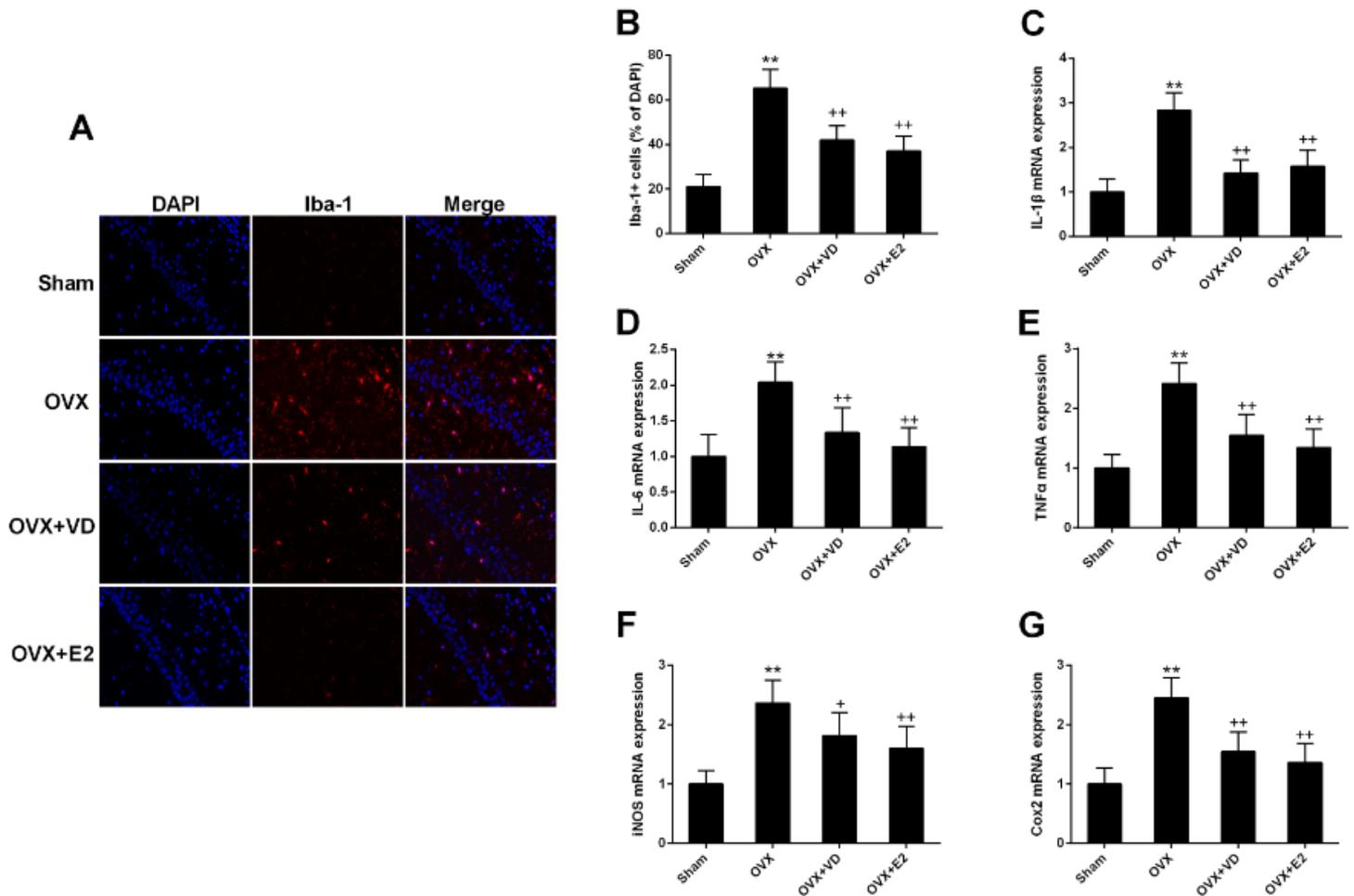


Figure 5

Anti-inflammatory effects of vitamin D and 17 β -estradiol in the hippocampus of OVX rats. Representative images of immunofluorescence assays of Iba-1 in the hippocampal CA1 region (A). Statistical graphs of Iba-1 positive cells (B). Relative mRNA expression of IL-1 β (C), IL-6 (D), TNF α (E), iNOS (F) and Cox2 (G). Data are the means \pm SD (n=7-8). * P < 0.05, ** P < 0.01 compared to the SHAM group. + P < 0.05, ++ P < 0.01 compared to the OVX group.