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Modulating Effects of Cholecalciferol Treatment on Estrogen Deficiency-Induced Anxiety-Like Behavior of Adult Female Rats

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Background: Vitamin D can be one of the candidate substances that are used as additional supplementation in the treatment of anxiety-related disorders in women with estrogen imbalance.

Materials and methods: The aim of the present study was to examine the effects of chronic cholecalciferol administration (1.0, 2.5 or 5.0 mg/kg/day, s.c.) on the anxiety-like behavior and monoamines levels in the rat hippocampus following ovariectomy in female rats. Cholecalciferol was given to ovariectomized (OVX) rats and OVX rats treated with 17 β -estradiol (17 β -E₂, 0.5 μ g/rat, s.c.). The anxiety-like behavior was assessed in the elevated plus maze (EPM) and the light-dark tests (LDT), locomotor and grooming activities were assessed in the open-field test (OFT).

Results: Cholecalciferol in high doses alone or in combination with 17 β -E₂-induced anxiolytic-like effects in OVX and OVX rats treated with 17 β -E₂ as evidenced in the EPM and LDT tests, and increased grooming activity in the OFT test. We found that DA and 5-HT levels increased while 5-HT turnover in the hippocampus decreased in these groups of OVX rats.

Conclusion: Our results indicate that cholecalciferol in high doses has a marked anxiolytic-like effect due to an increase in the monoamines levels in the experimental rat model of estrogen deficiency.

Abbreviations used in this article

CNS: central nervous system; DA: dopamine; DOPAC: 3,4-hydroxyphenylacetic acid; EPM: elevated plus maze; HPG system: hypothalamic-pituitary-gonadal system; HPLC: high pressure liquid chromatographic; LDT: light-dark test; MHPG: 3-methoxy-4-hydroxyphenylethylene glycol; NE: norepinephrine; OFT: open field test; OVX: ovariectomized; VD: vitamin D; 17 β -E₂: 17 β -estradiol; 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine, serotonin.

BACKGROUND

Anxiety- and stress-related disorders are one of the world's biggest mental health problems. Anxiety has a considerable economic impact on society as well, being associated with greater use of health care services^{1,2} and decreased work productivity^{3,4}. A large number of studies have also shown that anxiety-related disorders are often associated with

the quality of life impairments. Importantly, studies have reported that about 40% of patients diagnosed with anxiety and related disorders are often left untreated.^{4,5} Moreover, there exist gender differences in the development and manifestation of anxiety-related disorders.^{6,7} Anxiety-related disorders are twice as common in women as in men.^{7,8}

Hormonal mechanisms, especially the dysregula-

tion of the hypothalamic-pituitary-gonadal (HPG) axis, underlying affective-related disorders development, have gained renewed interest. Nowadays, the significance of gonadal hormones in the development of anxiety-like state has been extensively investigated.^{9,10} Estrogens have been shown to affect brain regions known to be involved in the modulation of mood and affective-related behavior. Several studies have identified a relationship between low estrogen levels and increased risk of anxiety disorders in women.⁶⁻⁸ Women with low estrogen levels have a significantly higher prevalence of mood- and stress-related disorders compared to those with normal physiological levels of gonadal steroids.^{6,7}

Clinical studies suggest that pathological affective-related disorders in women with estrogen deficiency can be reversed by chronic 17 β -estradiol administration.^{11,12} Estrogens have been shown to possess anxiolytic-like effects in several animal models of anxiety, reducing anxiety-like behavior in female rodents.^{6,13,14} Moreover, 17 β -estradiol replacement in ovariectomized females ameliorates anxiety-like behavior.^{7,14,15} Taken together, these data indicate a close relationship between anxiety-related disorders and low estrogen levels in women.

Vitamin D (VD) can be one of such candidate substances that can be used as additional supplementation for treatment of anxiety-related disorders in women with estrogen imbalance. VD has many physiological roles.^{16,17} In addition to its classic role in bone metabolism, VD may also have many potential non-skeletal functions.¹⁸⁻²⁰ This is supported by the finding that VD receptors are expressed not only in cells related to calcium and phosphate metabolism, but also in other cells and tissues in the human body.²¹ VD is becoming increasingly recognized as a neuroactive steroid affecting brain development and function.^{19,20} It has been shown that VD is involved in numerous brain processes including neurotrophism, neuroimmunomodulation and neurotransmission.^{21,22} Multiple lines of evidence suggest that VD is implicated in a number of neuropsychiatric diseases including Parkinson's disease, multiple sclerosis, schizophrenia and mood disturbances.^{23,24} Thus, supplementation with VD is implicated as a promising therapeutic strategy for the prevention or the treatment of these brain-related disorders, especially for treatment of anxiety-related disorders in women with estrogen deficiency.

Based on both evidence of estrogen involvement in the etiology of anxiety disorders and on our previous study showing a mood-enhancing effect

of a low dose of 17 β -estradiol in female rats with estrogen deficiency, we hypothesized that vitamin D₃ treatment alone or in a combination with low dose of 17 β -estradiol would modify the anxiety-like behavior of female rats with estrogen deficiency.

The aim of the present study was to evaluate the effects of chronic cholecalciferol administration on anxiety-related behavior in the adult female rats with estrogen deficiency. To test the hypothesis that different vitamin D₃ hormone levels may reverse anxiety-like behavior in the adult ovariectomized rats, female rats were treated with low, medium or high doses of cholecalciferol for 14 days. On the basis of these considerations, the purpose of this study was to characterize the anxiolytic-like activity of cholecalciferol on rat anxiety-like behavior and compared results with the effects of diazepam. The diazepam (as a benzodiazepine) is a standard anxiolytic and is also employed in behavioral pharmacology.^{25,26} Benzodiazepines have been the most extensively used anxiolytics for many years.²⁷ Diazepam is known to be anxiolytic in humans and reduced anxiety-like behavior in several animal models of behavior.²⁸ Furthermore, the biochemical effects of chronic cholecalciferol administration in the central nervous system (CNS) have not yet been determined, therefore, it would be important to conduct experimental studies to clarify its brain mechanism of action. Dopamine, serotonin, norepinephrine and their metabolites 3,4-hydroxyphenylacetic acid, homovanillic acid, 3-methoxy-4-hydroxyphenylethylene glycol, and 5-hydroxyindoleacetic acid represent the most important monoamines and metabolites derived from neurotransmitters. Such monoamines are the most abundant excitatory monoamines neurotransmitter and are widely distributed in the mammalian brain, including the hippocampus.²⁹ In this connection, the present work was also aimed to determine monoamines and their metabolites contents in rat hippocampus after chronic cholecalciferol administration, and to identify its mechanism of action in the CNS.

MATERIALS AND METHODS

ANIMALS

The study used 120 female Wistar rats (purchased from Rappolovo, Russia) weighing 180-200 g at the start of the experiment. For at least a week prior to the experiment, the rats were housed six to a cage under standard environmental conditions: constant temperature of 23 $\pm$ 1°C, 60% humidity,

12-h light/dark cycle (light on at 8:00 a.m.), food and water ad libitum. All animals were gently handled by experienced animal facility staff each day for a week prior to experimental procedures. Any environmental or physical stress was avoided in order to habituate the rats to manipulation. Animals were randomly assigned to experimental groups and were used only once in the behavioral experiments. The behavioral tests were conducted between 09:00 a.m. and 01:00 p.m. Experiments were carried out in a soundproof and air-regulated experimental room, to which animals were habituated at least 30 min before each test. All experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals, published by the National Institute of Health (National Research Council, publication no. 85-23, revised in 1996), and the Animal Welfare Assurance Renewal for Pavlov Institute of Physiology. The rationale, design, and methods of this study were approved by the Ethical Committee for Animal Research, Pavlov Institute of Physiology.

OVARIECTOMY

The female rats were anesthetized with a mixture of ketamine/xylazine (ketamine: 70 mg/kg b.w. and xylazine: 10 mg/kg b.w., i.p.) and bupivacaine (0.25% solution: 0.4 ml/kg b.w.) was applied topically as analgesic. The non-steroidal anti-inflammatory drug meloxicam (1 mg/kg b.w.) was injected subcutaneously. Following disinfection of the skin (with alcohol and Betadine), a dorsal midline skin incision was made caudal to the posterior of the ribs. Using blunt dissection to tunnel subcutaneously, lateral to the skin incision, the muscles of the posterior abdominal wall were separated in order to expose the abdominal cavity. The ovary is 1-2 cm located in a fat pad beneath the muscles. The periovarian fat was grasped to lift and exteriorize the ovary. The fallopian tube was crushed and the ovary was removed by cutting above the clamped area. The skin incision was closed using wound clips. Animal Welfare Assurance Renewal for Pavlov Institute of Physiology oversaw the entire surgical process, including post-operative care prior to shipment. The effectiveness of castration or exogenous administration of 17β -estradiol (17β -E₂) was controlled by vaginal smears.¹⁵ Following ovariectomy, ovariectomized (OVX) females were placed in a community cage with free access to food and water. OVX female rats were allowed to have 14 days for postoperative recovery before administration of solvent, cholecalciferol or 17β -E₂.

DRUGS

The estrogen, 17β -E₂ (E-8875, Sigma Chemical Co) was dissolved in sterile sesame oil. Cholecalciferol (C-9756, Sigma Chemical Co) was dissolved in 95% ethanol, aliquoted and stored at -80°C. The stock of cholecalciferol was diluted in a sterile water, resulting in a solution of cholecalciferol with 2% ethanol. 17β -E₂ was injected subcutaneously at a dose of 5.0 µg/rat. Ovariectomy markedly decreases estrogen level and 17β -E₂ receptor activity in the different structures of the brain.^{30,31} In this connection, a low dose of 17β -E₂ may play a trigger role in activation of 17β -E₂ receptors at the hypoestrogenic syndrome. Thus, we used a low dose of 17β -E₂ in our present study. The low dose of 17β -E₂ (5.0 µg/rat, s.c.) was chosen from the studies performed by Estrada-Camarena et al.^{32,33} Cholecalciferol was injected subcutaneously at three different doses (1.0, 2.5 or 5.0 mg/kg/day). Three doses (low, middle and high) of cholecalciferol were chosen from the behavioral study performed by Idrus et al.³⁴

Diazepam (D-094000, Sigma-Aldrich) was diluted with deionized water containing 0.5% propylene glycol (Sigma-Aldrich). Diazepam was administered intraperitoneally at a dose of 1.0 mg/kg.³⁵

All solutions were freshly prepared before each experimental series. All preparations were administered in a volume of 0.1 ml. After ovariectomy, diazepam, cholecalciferol, 17β -E₂ and oil solvent were injected once daily for 14 days. All females were 3.5 months old at the onset of pharmacological treatments.

EXPERIMENTAL GROUPS

In our previous studies (data not shown), we did not find any significant differences between control intact (sham-operated) rats treated with oil solvent, intact (sham-operated) females treated with deionized water containing 0.5% propylene glycol as solvent for diazepam, and intact (sham-operated) females treated with sterile water with 2% ethanol as solvent for cholecalciferol in behavioral tests for measurement of anxiety-like state, and biochemical measurements (data not shown). Since we did not find any differences between control groups of intact females with oil solvent, solvent for diazepam and solvent for cholecalciferol, we used only one control intact (sham-operated) group with oil solvent.

Two weeks after ovariectomy, OVX female rats were randomly assigned to each of the experimental groups and subjected to the respective treatments. All female OVX and intact rats were divided into

12 groups (n=8 per group) for each behavioral tests. The first and second group consisted of intact (sham-operated) female rats (control) daily treated with oil solvent (control/solvent) and intact (sham-operated) female rats daily treated with diazepam (intact/diazepam). The three other groups were of intact (sham-operated) female rats - they received daily a low dose of cholecalciferol (1.0 mg/kg, s.c.) (intact/cholecalciferol in low dose), a middle dose of cholecalciferol (2.5 mg/kg, s.c.) (intact/cholecalciferol in a middle dose) or a high dose of cholecalciferol (5.0 mg/kg, s.c.) (intact/cholecalciferol in a high dose). The next four groups were of OVX female rats - they received the oil solvent daily (OVX/solvent), OVX female rats daily treated with diazepam (OVX/diazepam), OVX rats treated with 17 β -E₂ at a daily dose of 5.0 μ g/rat, s.c. (OVX/17 β -E₂) and OVX rats treated with 17 β -E₂ with diazepam (OVX/17 β -E₂/diazepam). The other groups consisted of the OVX female rats treated with low dose of cholecalciferol (1.0 mg/kg, s.c.) (OVX/cholecalciferol in low doses), OVX female rats treated with a middle dose of cholecalciferol (2.5 mg/kg, s.c.) (OVX/cholecalciferol in middle doses), OVX female rats treated with a high dose of cholecalciferol (5.0 mg/kg, s.c.) (OVX/cholecalciferol in high dose), OVX female rats treated with low dose of cholecalciferol and 17 β -E₂ (OVX/cholecalciferol in low dose/17 β -E₂), OVX female rats treated with low dose of cholecalciferol and 17 β -E₂ (OVX/cholecalciferol in middle doses/17 β -E₂), OVX female rats treated with low dose of cholecalciferol and 17 β -E₂ (OVX/cholecalciferol in high doses/17 β -E₂). All experimental groups are shown in **Table 1**.

To summarize the treatment workflow, after induction of the experimental model of estrogen deficiency, the rats were left to recover for 2 weeks. After that time, the rats received daily injections for 14 days with either diazepam, cholecalciferol, 17 β -E₂ or oil solvent. One hour after the last injection, testing in the elevated plus maze test, the light-dark test and the open field test was carried out as described below. During all behavioral tests the control (sham-operated) and experimental groups of rats were also treated with cholecalciferol, 17 β -E₂ or solvent.

BEHAVIORAL TESTS

Before testing, animals were handled daily for 1 week. Behavioral experiments were carried out in a soundproof and air-regulated experimental room to which animals were habituated at least 30 min

Table 1. Experimental groups of animals

Experimental groups of animals

Intact sham-operated rats + solvent (control/solvent rats)
Intact sham-operated rats + diazepam (0.5 mg/kg, i.p.) (intact/diazepam rats)
Intact sham-operated rats + cholecalciferol (1.0 mg/kg, s.c.) (intact/cholecalciferol in a low dose rats)
Intact sham-operated rats + cholecalciferol (2.5 mg/kg, s.c.) (intact/cholecalciferol in a middle dose rats)
Intact sham-operated rats + cholecalciferol (5.0 mg/kg, s.c.) (intact/cholecalciferol in a high dose rats)
OVX rats + solvent (OVX/solvent rats)
OVX rats + diazepam (OVX/diazepam rats)
OVX rats + 17 β -estradiol (5.0 μ g/rat, s.c.) (OVX/17 β -E ₂ rats)
OVX rats + 17 β -estradiol + diazepam (OVX/17 β -E ₂ /diazepam rats)
OVX rats + cholecalciferol (1.0 mg/kg, s.c.) (OVX/cholecalciferol in a low dose rats)
OVX rats + cholecalciferol (2.5 mg/kg, s.c.) (OVX/cholecalciferol in a middle dose rats)
OVX rats + cholecalciferol (5.0 mg/kg, s.c.) (OVX/cholecalciferol in a high dose rats)
OVX rats + cholecalciferol (1.0 mg/kg, s.c.) + 17 β -estradiol (OVX/cholecalciferol in a low dose + 17 β -E ₂ rats)
OVX rats + cholecalciferol (2.5 mg/kg, s.c.) + 17 β -estradiol (OVX/cholecalciferol in a middle dose + 17 β -E ₂ rats)
OVX rats + cholecalciferol (5.0 mg/kg, s.c.) + 17 β -estradiol (OVX/cholecalciferol in a high dose + 17 β -E ₂ rats)

before each test. Any environmental or physical stress was avoided in order to habituate the rats to manipulation for behavioral tests. The apparatus used in all behavioral experiments were thoroughly cleaned after each test session with a cleaning solution from Vekton (Russia, with a composition of ammonia 0.5%, ethanol 15%, extran 10%, isopropyl alcohol 5%, citrus aromatizing 19%, and distilled water 50.5% as v/v%).

ELEVATED PLUS MAZE TEST

To investigate the changes in anxiety-like behavior, control intact (sham-operated) rats and all experimental groups of OVX female rats were subjected to the elevated plus maze test (EPM).²⁸ EPM is a widely used test of anxiety-like behavior and was used to assess an anxiety-like behavioral responses.³⁶ This test is sensitive to putative anxiogenic-like and anxiolytic-like drugs.³⁷ It is designed to present the animal with a conflict between its natural tendency to explore a novel environment and its reluctance to move away from the sheltering walls and into the open environment in which the risk of falling or exposure to predators is much higher. The maze was made of grey Plexiglas and consisted of four arms (50 cm long and 10 cm wide); two arms had 40-cm-high dark walls (closed arms), and two arms had 0.5-cm-high ledges (open arms). In the center of the arms of EPM located cross-wise there was an open area 10×10 cm. The floor of the apparatus was 50 cm high. The experimental room was lit by a 60-watt bulb placed 1.75 m above the central square of the maze (22 lx in the maze central square). For the testing, rats were placed individually into the center of the maze facing a closed arm and removed after a 5-min period. The number of entrances and the time spent into the open or closed arms were registered during time of testing. A video camera was installed above the cage to record the activity of the rats. Two independent observers measured the behavioral variables. After each test session, the EPM apparatus was carefully cleaned and deodorized with the Vekton cleaning solution.

LIGHT/DARK TEST

The light/dark test (LDT) was used to test unconditioned anxiety and exploratory behavior. It is based on the natural aversion of rodents to bright light in novel environments.^{38,39} The apparatus was consisted of a Plexiglas box with two equal compartments (30×40×40 cm), one of which with white walls and floor and illuminated by a 60-watt light from above, while the other of the boxes was painted black and

had a lid so it was not illuminated. Each animal was placed at the junction of the light/dark, facing the illuminated compartment. The time spent and the number of entrances in the illuminated compartment were recorded for 5 min.⁴⁰

Increased time in the light side is indicative of anti-anxiety behavior. A video camera was installed above the cage to record the activity of the rats. Two independent observers measured the behavioral variables. After each test session, the OFT apparatus was carefully cleaned and deodorized with the Vekton cleaning solution.

OPEN FIELD TEST

To investigate the changes in spontaneous locomotor activity, grooming, and rearing, all experimental groups of offspring were submitted to a 5-min period to the open field test (OFT) as described previously.⁴¹ Two independent observers (blind to treatment groups) measured the behavioral variables. A video camera was installed above the cage to record the activity of the rats. After each test session, the OFT apparatus was carefully cleaned and deodorized with the Vekton cleaning solution.

ESTIMATION OF RAT BRAIN MONOAMINES AND METABOLITES BY HPLC ASSAY

Monoamines and their main metabolites of 5-HT, 5-HIAA, DA, HVA, DOPAC, NA and MHPG levels were identified and quantified using a HPLC technique with electrochemical detector. Hippocampus was removed, placed on an ice chilled plate, weighed and stored at -80°C until assay. Tissue was ultrasonically homogenized in a 0.1 mol/L solution of HClO₄ containing 0.02% Na₂S₂O₂ (15 µL of solution for each milligram of tissue), dihydroxybenzylamine (146.5 ng/mL), as the internal standard for monoamines. The samples were centrifuged at 11000 g at 4°C for 40 min, and then the supernatant was filtered and injected into an HPLC system. The monoamines NA, DA and 5-HT, as well as their metabolites, were quantified as described by Cavalheiro et al.⁴² In summary, a Shimadzu LC-10AD isocratic system was employed, with a 20 µL injection loop and a Spheri-5 RP-18 5 µm column (220 × 4.6 mm), using electrochemical detection at 0.75 V and a mobile phase composed of phosphate/citrate pH 2.64, 0.02 mol/L, 0.12 mmol/L ethylene diamine tetra-acetic acid and 0.06% heptane sulphonic acid, in 10% methanol, at a flow rate of 1 mL/min. Concentrations of monoamines and metabolites were expressed as mean ± SD ng/mg wet tissue. Moreover, the ratio between monoamines and their metabolites concen-

trations were calculated. Before sample analysis, a solution was prepared with 1 mL of stock solution and 2 mL of sodium tetraborate 0.1 mol/L. The pre-column derivatization was completed by reacting 100 μ L of this solution with 50 μ L of sample for 2 min before the injection.⁴³ The mobile phase consisted of sodium phosphate 0.05 mol/L (pH 5.95) with methanol 11.5%. The flow rate of this HPLC system was 3.5 mL/min and the detector was employed with an excitation of 348 nm and emission of 460 nm. Standard concentrations of monoamines were tested and the retention time was verified for each substance to certify that there were no peaks overlapping on sample delivery.

STATISTICAL ANALYSIS

All values were expressed as mean $\pm$ S.E.M. Comparisons between values were performed using two-way ANOVA with between subject factors for hormone state (OVX or OVX plus 17β -E₂) and drug treatments followed by Dunnett's test for multiple comparisons post-hoc test. Statistical analysis was performed using SPSS version 11.5.

RESULTS

EFFECTS OF CHOLECALCIFEROL ADMINISTRATION ON ANXIETY-LIKE BEHAVIOR OF OVX AND OVX RATS TREATED WITH 17β -ESTRADIOL IN THE ELEVATED PLUS MAZE

A two-way ANOVA revealed significant differences in the time spent into the open arms between hormone conditions ($F(5,32) = 7.4$, $p < 0.0001$), between drug treatments ($F(5,32) = 11.04$, $p < 0.0001$), and an interaction between hormone condition and treatments ($F(5,32) = 1.84$, $p < 0.001$) in the GDX rats with A β 25-35-induced anxiety. The post-hoc test revealed differences among the groups for anxiety-like behavior in the EPM ($p < 0.05$).

The intact/diazepam rats demonstrated a significant increase of the time spent into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**). The intact/cholecalciferol in low and intact/cholecalciferol in middle doses female rats failed to modify the time spent into the open arms as compared to the control/solvent rats ($p > 0.05$, **Table 2**). The intact/cholecalciferol in high dose rats showed a significant increase of the time spent into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**).

Ovariectomy in female rats (OVX/solvent rats) resulted in a significant decrease of the time spent into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**). The OVX/diazepam

rats demonstrated a significant increase of the time spent into the open arms as compared to the OVX/solvent rats ($p < 0.05$, **Table 2**). 17β -E₂ supplementation (0.5 μ g/kg, s.c.) caused an increase of the time spent into the open arms in the OVX rats as compared to the OVX/solvent rats ($p < 0.05$, **Table 2**). The OVX/ 17β -E₂/diazepam rats demonstrated a significant increase of the time spent into the open arms as compared to the OVX/solvent rats ($p < 0.05$, **Table 2**). Although, the value of these parameters in the OVX/diazepam, OVX/ 17β -E₂ and OVX/ 17β -E₂/diazepam female rats was higher than that of the OVX/solvent rats, it did not reach the value of control sham-operated rats (**Table 2**).

The OVX/cholecalciferol in low and in middle doses rats did not show any changes of the time spent into the open arms as compared to the OVX/solvent rats ($p > 0.05$, **Table 2**). The OVX/cholecalciferol in low and middle doses/ 17β -E₂ demonstrated a significant increase of the time spent into the open arms as compared to the OVX/solvent rats, like OVX rats treated only with 17β -E₂ alone ($p < 0.05$, **Table 2**). Administration of cholecalciferol in high doses (5.0 mg/kg/day, s.c.) significantly increased the time spent into the open arms in the OVX rats as compared to the OVX/solvent ($p < 0.05$, **Table 2**). Cholecalciferol in high doses (5.0 mg/kg/day, s.c.) in combination with 17β -E₂ increased more significantly the time spent into the open arms in the OVX rats as compared to the OVX rats treated with oil solvent or 17β -E₂ ($p < 0.05$, **Table 2**). Moreover, the time spent into the open arms values of the OVX rats given with combination of cholecalciferol in a high dose (5.0 mg/kg/day, s.c.) and 17β -E₂ were much higher than that of the OVX/ 17β -E₂ rats.

Similarly, significant differences in the number of entries into the open arms were found between hormone conditions ($F(5,32) = 2.96$, $p < 0.01$), between drug treatments ($F(5,32) = 7.20$, $p < 0.001$), and an interaction between hormone condition and treatments ($F(5,32) = 11.22$, $p < 0.0001$) in the OVX rats. The post-hoc test revealed differences among the groups for anxiety-like behavior in the EPM ($p < 0.05$). The intact/diazepam rats demonstrated a significant increase of the number of entries into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**). The intact/cholecalciferol in low doses and intact/cholecalciferol in middle doses female rats failed to alter the number of entries into the open arms as compared to the control/solvent rats ($p > 0.05$, **Table 2**). The intact/cholecalciferol in high doses showed a significant increase of the

number of entries into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**). OVX/solvent and OVX/diazepam rats displayed a significant decrease of the number of entries into the open arms as compared to the control/solvent rats ($p < 0.05$, **Table 2**). Administration of 17β -E₂ to the OVX rats increased the number of entries into the open arms as compared to the OVX/solvent rats ($p < 0.05$, **Table 2**). The OVX/ 17β -E₂/diazepam female rats showed an increase of the number of entries into the open arms as compared to the OVX/solvent rats, like OVX/ 17β -E₂ group of rats ($p < 0.05$, **Table 2**). Although the value of these parameters in the OVX/ 17β -E₂ and OVX/ 17β -E₂/diazepam female rats was higher than that of the OVX/solvent rats, it did not reach the value of control sham-operated rats (**Table 2**).

The OVX/cholecalciferol in low and in middle doses rats failed to show any modifications of the number of entries into the open arms as compared to the OVX/solvent rats ($p > 0.05$, **Table 2**). The OVX/cholecalciferol in low and middle doses/ 17β -E₂ rats demonstrated significant increase of the number of entries into the open arms as compared to the OVX/solvent rats, like OVX rats treated only with 17β -E₂ alone ($p < 0.05$, **Table 2**). Administration of cholecalciferol in high doses (5.0 mg/kg/day, s.c.) induced increase of the number of entries into the open arms in the OVX rats as compared to the OVX/solvent ($p < 0.05$, **Table 2**). Cholecalciferol (5.0 mg/kg/day, s.c.) in combination with 17β -E₂ more significantly increased the number of entries into the open arms in the OVX rats as compared to the OVX rats treated with oil solvent or 17β -E₂ ($p < 0.05$, **Table 2**). Furthermore, there was a significant increase of the number of entries into the open arms values of the OVX rats given with combination of cholecalciferol in high dose (5.0 mg/kg/day, s.c.) and 17β -E₂ as compared to the OVX/ 17β -E₂ rats.

EFFECTS OF CHOLECALCIFEROL ADMINISTRATION ON ANXIETY-LIKE BEHAVIOR OF OVX AND OVX RATS TREATED WITH 17β -ESTRADIOL IN THE LIGHT-DARK TEST

The two-way ANOVA showed significant differences in the time spent and number of entries in the light compartment between hormone conditions ($F(5,32) = 11.92$, $p < 0.05$) and ($F(5,32) = 22.11$, $p < 0.01$), between drug treatments ($F(5,32) = 7.18$, $p < 0.001$) and ($F(5,32) = 7.26$, $p < 0.05$), and an interaction between hormone condition and treatments ($F(5,32) = 5.42$, $p < 0.01$) and ($F(5,32) = 14.46$, $p < 0.05$) in

the OVX rats. The post-hoc test revealed differences among the groups for anxiety-like behavior in the LDT ($p < 0.05$).

The intact/diazepam rats demonstrated a significant increase of time spent and number of entries in the light box as compared to the control/solvent rats ($p < 0.05$, **Table 3**). The intact/cholecalciferol in low and intact/cholecalciferol in middle doses female rats failed to modify the time spent and number of entries in the light box as compared to the control/solvent rats ($p > 0.05$, **Table 3**). The intact/cholecalciferol in high dose demonstrated a significant increase of the time spent and number of entries in the light box as compared to the control/solvent rats ($p < 0.05$, **Table 3**).

Ovariectomy in female rats (OVX/solvent rats) resulted in a significant decrease of the time spent and number of entries in the light box as compared to the control/solvent rats ($p < 0.05$, **Table 3**). 17β -E₂ supplementation (0.5 μ g/kg, s.c.), diazepam treatment or co-administration of 17β -E₂ and with diazepam caused an increase of the time spent and number of entries in the light box in the OVX rats as compared to the OVX/solvent rats ($p < 0.05$, **Table 3**). Although, the value of these parameters in the OVX/ 17β -E₂, OVX/diazepam and OVX/ 17β -E₂/diazepam females were higher than that of the OVX/solvent, it did not reach the value of control/solvent rats.

The OVX/cholecalciferol in low and middle doses rats did not show any changes of the time spent and number of entries in the light box as compared to the OVX/solvent rats ($p < 0.05$, **Table 3**). The OVX/cholecalciferol in low and in middle dose/ 17β -E₂ rats demonstrated significant increase of the time spent and number of entries in the light box as compared to the OVX/solvent rats, like OVX rats treated only with 17β -E₂ alone ($p < 0.05$, **Table 3**). Administration of cholecalciferol in high dose (5.0 mg/kg/day, s.c.) significantly increased the time spent and number of entries in the light box in the OVX rats as compared to the OVX/solvent ($p < 0.05$, **Table 3**). Cholecalciferol (5.0 mg/kg/day, s.c.) in combination with 17β -E₂ increased more significantly the time spent and number of entries in the light box in the OVX rats as compared to the OVX rats treated with oil solvent or 17β -E₂ ($p < 0.05$, **Table 3**). Moreover, the time spent and number of entries in the light box values of the OVX rats given with combination of cholecalciferol in high dose (5.0 mg/kg/day, s.c.) and 17β -E₂ were much higher than that of the OVX/ 17β -E₂ rats.

EFFECTS OF CHOLECALCIFEROL ADMINISTRATION ON BEHAVIORAL IMPAIRMENTS OF OVX AND OVX RATS TREATED WITH 17 β -ESTRADIOL IN THE OPEN FIELD TEST

The two-way ANOVA revealed significant differences in the crossing, rearing and grooming behaviors between hormone conditions ($F(5,32) = 5.44$, $p < 0.05$), ($F(5,32) = 9.40$, $p < 0.01$), ($F(5,32) = 19.34$, $p < 0.01$), between drug treatments ($F(5,32) = 15.4$, $p < 0.001$), ($F(5,32) = 11.56$, $p < 0.05$), ($F(5,32) = 7.86$, $p < 0.05$), and an interaction between hormone condition and treatments ($F(5,32) = 3.8$, $p < 0.01$), ($F(5,32) = 5.46$, $p < 0.05$), ($F(5,32) = 4.02$, $p < 0.05$), in the OVX rats. The post-hoc test revealed differences among the groups for behavior in the OFT ($p < 0.05$).

The intact female rats treated with diazepam showed a significant decrease of crossing, rearing and grooming behaviours as compared to the control/solvent rats ($p < 0.05$, **Table 4**). The sham-operated female rats treated with cholecalciferol in low dose demonstrated a significant decrease of grooming as compared to the control/solvent rats ($p < 0.05$, ($p < 0.05$, **Table 4**). The post-hoc test failed to reveal any alterations of behavioral values in the intact/cholecalciferol in middle and intact/cholecalciferol in high doses rats as compared to the intact/solvent rats (**Table 4**, non-significant).

OVX/solvent rats demonstrated a significant decrease of grooming behavior as compared to the control/solvent rats ($p < 0.05$, **Table 4**). The administration of diazepam to the OVX rats resulted in a significant decrease of crossing, rearing and grooming behaviours as compared to the OVX/solvent and control/solvent rats ($p < 0.05$, **Table 4**). 17 β -E₂ Supplementation produced a significant increase of grooming reactions when OVX/17 β -E₂ rats were compared to the OVX/solvent rats ($p < 0.05$, **Table 4**). The OVX/17 β -E₂/diazepam rats significantly demonstrated increase of crossing, rearing and grooming behaviours as compared to the OVX/solvent rats ($p < 0.05$, **Table 4**). The post-hoc test failed to reveal any alterations of behavioral activities in the OVX rats treated with cholecalciferol in low and middle doses alone or in a combination with 17 β -E₂ as compared to the OVX/solvent or OVX/17 β -E₂ rats ($p > 0.05$, **Table 4**). Cholecalciferol in high doses (5.0 mg/kg/day, s.c.) significantly elevated frequency of grooming when the OVX/cholecalciferol in high doses rats were compared to OVX/solvent rats ($p < 0.05$, **Table 4**). Cholecalciferol in high dose given together with 17 β -E₂ resulted in a significant increase of frequency of grooming when OVX/cholecalciferol in high dose+17 β -E₂ rats

were compared to OVX/17 β -E₂ and OVX/solvent rats ($p < 0.05$, **Table 4**).

EFFECTS OF CHOLECALCIFEROL ADMINISTRATION ON MONOAMINES, THEIR METABOLITES LEVELS AND TURNOVER RATES IN THE HIPPOCAMPUS OF OVX AND OVX RATS TREATED WITH 17 β -ESTRADIOL

A two-way ANOVA revealed significant differences in dopamine, serotonin and noradrenaline levels between hormone conditions ($F(5,32) = 22.11$, $p < 0.01$), ($F(5,32) = 14.99$, $p < 0.05$), ($F(5,32) = 3.88$, $p < 0.05$), between drug treatments ($F(5,32) = 7.4$, $p < 0.05$), ($F(5,32) = 7.40$, $p < 0.01$), ($F(5,32) = 15.22$, $p < 0.05$) and an interaction between hormone condition and treatments ($F(5,32) = 17.4$, $p < 0.01$), ($F(5,32) = 16.5$, $p < 0.01$) and $F(5,32) = 10.8$, $p < 0.05$) in the GDX rats with A β 25-35 induced amnesia. The post-hoc test revealed differences among the DA, 5-HT and NA levels in the HPLC method ($p < 0.05$, $p < 0.05$ and $p < 0.01$). Moreover, a two-way ANOVA revealed significant differences in 5-hydroxyindole acetic acid, homovanillic acid, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylethylene glycol levels between hormone conditions ($F(5,32) = 14.02$, $p < 0.01$), ($F(5,32) = 11.58$, $p < 0.05$), ($F(5,32) = 10.6$, $p < 0.05$), ($F(5,32) = 15.22$, $p < 0.05$), between drug treatments ($F(5,32) = 7.4$, $p < 0.05$), ($F(5,32) = 9.40$, $p < 0.01$), ($F(5,32) = 9.44$, $p < 0.05$) and an interaction between hormone condition and treatments ($F(5,32) = 17.4$, $p < 0.01$), ($F(5,32) = 15.22$, $p < 0.05$), ($F(5,32) = 16.5$, $p < 0.01$) and $F(5,32) = 17.3$, $p < 0.05$) in the OVX/solvent rats. The post-hoc test revealed differences among the DOPAC, HVA, 3,4-MHPG and 5-HIAA contents in the HPLC method ($p < 0.05$, $p < 0.01$, $p < 0.05$ and $p < 0.01$). In addition, a two-way ANOVA revealed significant differences in DA, NA and 5-HT turnover rates between hormone conditions ($F(5,32) = 7.56$, $p < 0.01$, $F(5,32) = 15.22$, $p < 0.05$), ($F(5,32) = 12.34$, $p < 0.05$, ($F(5,32) = 11.56$, $p < 0.05$), between drug treatments ($F(5,32) = 7.8$, $p < 0.05$), ($F(5,32) = 7.40$, $p < 0.01$), ($F(5,32) = 15.22$, $p < 0.05$), ($F(5,32) = 10.8$, $p < 0.05$) and an interaction between hormone condition and treatments ($F(5,32) = 3.7$, $p < 0.01$), ($F(5,32) = 3.2$, $p < 0.01$), ($F(5,32) = 8.76$, $p < 0.05$ and $F(5,32) = 9.41$, $p < 0.05$) in the OVX/solvent rats. The post-hoc test revealed differences between the DA, NA and 5-HT turnover rates in the HPLC method ($p < 0.05$, $p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively).

The intact sham-operated rats given with diazepam did not show any alterations of monoamines and its metabolites levels, as well as turnover rates of the

monoamines in the hippocampus as compared to the control/solvent rats ($p > 0.05$, **Tables 5, 6**). The intact/cholecalciferol in low, intact/cholecalciferol in middle or intact/cholecalciferol in high doses female rats failed to demonstrate any modifications of monoamines and its metabolites levels, as well as turnover rates of the monoamines in the hippocampus as compared to the control/solvent rats ($p > 0.05$, **Tables 5, 6**).

The OVX female rats (OVX/solvent rats) demonstrated a significant decrease of DA, 5-HT and NA levels in the hippocampus compared to the control rats ($p < 0.05$, **Table 5**). In addition, the OVX/solvent rats showed a significant reduction of 5-HIAA, DOPAC and HVA contents in the hippocampus as compared to the control/solvent rats ($p < 0.05$, **Table 4**). We found a significantly higher DOPAC/DA, 3,4-MHPG/NA and 5-HIAA/5-HT ratios in the hippocampus of OVX/solvent rats as compared to the control/solvent rats ($p < 0.05$, **Table 5**). The monoamines and its metabolites concentrations, as well as turnover rates of the monoamines in the hippocampus of OVX/diazepam rats were not statistically different from OVX/solvent rats ($p > 0.05$, **Tables 5, 6**). Application of 17β -E₂ (0.5 μ g/kg, s.c.) produced a significant increase of DA, NA and 5-HT levels when OVX/ 17β -E₂ rats were compared with the of OVX/solvent rats ($p < 0.05$, **Table 5**). However, these parameters of monoamines levels in the OVX/ 17β -E₂ females were significantly lower than those in the control/solvent rats ($p < 0.05$, **Table 5**). The OVX/ 17β -E₂ rats showed a significant increase of DOPAC content in the hippocampus as compared to the of OVX/solvent rats ($p < 0.05$, **Table 5**). Content of 3,4-MHPG in the hippocampus of OVX/ 17β -E₂ rats was reduced as compared to the OVX/solvent and control/solvent rats ($p < 0.05$, **Table 5**). The HVA levels in OVX/ 17β -E₂ rats were significantly lower, while 5-HIAA contents in OVX/ 17β -E₂ rats were significantly higher as compared to the OVX/solvent and control/solvent rats ($p < 0.05$, **Table 5**). The NA turnover rate in the hippocampus of OVX/ 17β -E₂ rats were diminished as compared to the OVX/solvent rats ($p < 0.05$, **Table 6**). In addition, the 5-HIAA/5-HT ratio in the hippocampus of OVX/ 17β -E₂ rats was higher as compared to the OVX/control and control/solvent rats ($p < 0.05$, **Table 6**). The OVX/ 17β -E₂ rats showed a significantly decreased HVA/DA ratio compared to the OVX/solvent and control/solvent rats ($p < 0.05$, **Table 6**). The values of the monoamines and its metabolites levels, as well as the turnover rates of the monoamines in

the hippocampus of OVX/ 17β -E₂/diazepam rats as compared to the OVX/solvent and control/solvent rats ($p > 0.05$, **Tables 5, 6**).

The post-hoc test failed to reveal any alterations of monoamines and its metabolites levels, as well as turnover rates of the monoamines in the OVX/cholecalciferol in low and OVX/cholecalciferol in middle doses rats as compared to the OVX/solvent rats (**Tables 5, 6**).

Administration of cholecalciferol in high doses (5.0 mg/kg/day, s.c.) significantly increased DA and 5-HT levels in the hippocampus of OVX rats when OVX/cholecalciferol in a high dose rats were compared to the OVX/solvent rats ($p < 0.05$, **Table 5**). OVX/cholecalciferol in a high dose rats demonstrated a significant increase of DOPAC and HVA contents as compared with the OVX/solvent rats ($p < 0.05$, **Table 5**). In addition, treatment of cholecalciferol in high doses produced a pronounced decrease of 5-HIAA concentration as compared to the OVX/solvent rats ($p < 0.05$, **Table 5**). The DOPAC/DA and 5-HIAA/5-HT ratios in the hippocampus of OVX/cholecalciferol in high dose rats were decreased as compared to the OVX/solvent rats ($p < 0.05$, **Table 6**). However, the 3,4-MHPG/NA ratio in the hippocampus of OVX/cholecalciferol in a high dose rats did not differ from the OVX/solvent rats ($p > 0.05$, **Table 6**).

The OVX/cholecalciferol in low and in middle doses/ 17β -E₂ rats demonstrated the similar modifications of the monoamines, its metabolites and their turnover, like OVX rats treated only with 17β -E₂ alone ($p < 0.05$, **Tables 5, 6**). Cholecalciferol in high doses in combination with 17β -E₂ increased more significantly DA and 5-HT levels in the hippocampus when OVX/cholecalciferol in high dose/ 17β -E₂ rats were compared with the OVX/solvent group ($p < 0.05$, **Table 5**). The DA and 5-HT levels for OVX/cholecalciferol in high dose/ 17β -E₂ rats were markedly higher than that of OVX/ 17β -E₂ rats. OVX/cholecalciferol in high dose/ 17β -E₂ rats demonstrated a significant increase of DOPAC and HVA contents compared with the OVX/solvent rats ($p < 0.05$, **Table 5**). Moreover, cholecalciferol in a high dose administration in combination with 17β -E₂ resulted in a significant decrease of 5-HIAA in the hippocampus compared to the instance when OVX/cholecalciferol in high dose/ 17β -E₂ rats were compared with the OVX/ 17β -E₂ and OVX/solvent rat groups ($p < 0.05$, **Table 5**). Cholecalciferol in a high dose administration in combination with 17β -E₂ significantly decreased DOPAC/DA, HVA/DA,

and 5-HIAA/5-HT ratios in the hippocampus when OVX/cholecalciferol in high dose+17 β -E₂ rats were compared with the OVX/17 β -E₂ and OVX/solvent rats ($p < 0.05$, **Table 6**). The DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios for OVX/cholecalciferol in high dose+17 β -E₂ rats were markedly lower than that of OVX/17 β -E₂ rats. Cholecalciferol in high dose in combination with 17 β -E₂ caused a significant decrease of 3,4-MHPG in the hippocampus, like

DISCUSSION

We investigated the effects of chronic cholecalciferol treatment in different doses (low, middle and high) for 14 days on anxiety-like behavior in female rats with estrogen deficiency and 17 β -E₂ supplementation. Endogenous estrogens were removed by ovariectomy and the results of behavioral testing for the anxiety-related effects of cholecalciferol were compared in

Table 2. Effects of chronic cholecalciferol administration on anxiety-like behavior of OVX female rats and OVX female rats treated with 17 β -estradiol in the elevated plus-maze test

Groups	Time spent into the open arms	The number of entries into the open arms
Intact sham-operated rats + solvent (control/solvent rats)	76.1 $\pm$ 5.8	2.3 $\pm$ 0.4
Intact sham-operated rats + diazepam (intact/diazepam rats)	97.3 $\pm$ 6.2*	4.8 $\pm$ 0.2*
Intact/cholecalciferol in a low dose rats	73.5 $\pm$ 8.4	1.8 $\pm$ 0.2
Intact/cholecalciferol in a middle dose rats	80.2 $\pm$ 6.6	1.5 $\pm$ 0.4
Intact/cholecalciferol in a high dose rats	109.3 $\pm$ 10.2*	4.1 $\pm$ 0.6*
OVX rats + solvent (OVX/solvent rats)	35.0 $\pm$ 12.2*	0.6 $\pm$ 0.2*
OVX rats + diazepam (OVX/diazepam rats)	51.7 $\pm$ 6.4*	0.5 $\pm$ 0.2*
OVX rats + 17 β -E ₂ (OVX/17 β -E ₂ rats)	58.3 $\pm$ 10.4* [#]	1.1 $\pm$ 0.4* [#]
OVX rats + 17 β -estradiol + diazepam (OVX/17 β -E ₂ /diazepam rats)	60.4 $\pm$ 4.8* [#]	1.2 $\pm$ 0.2* [#]
OVX/cholecalciferol in a low dose rats	37.8 $\pm$ 13.4*	0.8 $\pm$ 0.2*
OVX/cholecalciferol in a middle dose rats	40.1 $\pm$ 12.5*	0.5 $\pm$ 0.2*
OVX/cholecalciferol in a high dose rats	69.8 $\pm$ 14.2 [#]	2.0 $\pm$ 0.4 [#]
OVX/cholecalciferol in a low dose + 17 β -E ₂ rats	66.2 $\pm$ 12.4 [#]	1.0 $\pm$ 0.4 [#]
OVX/cholecalciferol in middle dose + 17 β -E ₂ rats	57.8 $\pm$ 12.7 [#]	1.3 $\pm$ 0.6 [#]
OVX/cholecalciferol in a high dose + 17 β -E ₂ rats	103.4 $\pm$ 12.6* [#] ^{###}	4.5 $\pm$ 0.6* [#] ^{###}

* $p < 0.05$ as compared to the control group of sham-operated rats, [#] $p < 0.05$ as compared to the OVX rats treated with solvent, ^{###} $p < 0.05$ as compared to the OVX rats treated with 17 β -estradiol. Each group comprised a minimum of 8 rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. 17 β -Estradiol (17 β -E₂) was given at 0.5 μ g/rat, s.c., once daily, for 14 days. The obtained results show the mean $\pm$ S.E.M.

17 β -E₂, compared to the when OVX/cholecalciferol in a high dose+17 β -E₂ rats were compared with the OVX/solvent rat ($p < 0.05$, **Table 5**). The NA turnover rate in the hippocampus of OVX/cholecalciferol in high dose+17 β -E₂ rats were lower compared to the OVX/solvent rats ($p < 0.05$, **Table 6**).

both OVX rats and OVX female rats treated with 17 β -E₂. Simultaneously, effects of cholecalciferol treatment in similar doses on anxiety-like behavior were carried out in intact female rats. For this purpose, the elevated plus maze (EPM) and light-dark test (LDT) were performed in this study. The EPM and LDT are recognized as a valuable model able to

Table 3. Effects of chronic cholecalciferol administration on anxiety-like behavior of OVX females and OVX females treated with 17 β -estradiol in the light-dark test

Groups	Time spent in the light box	The number of entrances in the light box
Intact sham-operated rats + solvent (control/solvent rats)	176.7 $\pm$ 12.6	7.8 $\pm$ 0.6
Intact sham-operated rats + diazepam (Intact/diazepam rats)	205.2 $\pm$ 10.4*	13.1 $\pm$ 0.8*
Intact/cholecalciferol in a low dose rats	158.4 $\pm$ 14.4	8.2 $\pm$ 1.2
Intact/cholecalciferol in a middle dose rats	163.1 $\pm$ 12.6	9.2 $\pm$ 0.8
Intact/cholecalciferol in a high dose rats	215.9 $\pm$ 10.6*	13.9 $\pm$ 0.8*
OVX rats + solvent (OVX/solvent rats)	102.7 $\pm$ 12.4*	2.4 $\pm$ 0.6*
OVX rats + diazepam (OVX/diazepam rats)	152.7 $\pm$ 8.5*	4.9 $\pm$ 0.2*
OVX rats + 17 β -E ₂ (OVX/17 β -E ₂ rats)	138.1 $\pm$ 10.4* [#]	5.0 $\pm$ 0.4* [#]
OVX rats + 17 β -estradiol + diazepam (OVX/17 β -E ₂ /diazepam rats)	144.3 $\pm$ 6.7* [#]	5.1 $\pm$ 0.4* [#]
OVX/cholecalciferol in a low dose rats	105.2 $\pm$ 12.5*	2.2 $\pm$ 0.6*
OVX/cholecalciferol in a middle dose rats	100.8 $\pm$ 14.3*	2.1 $\pm$ 0.8*
OVX/cholecalciferol in a high dose rats	166.7 $\pm$ 14.2 [#]	6.9 $\pm$ 0.4 [#]
OVX/cholecalciferol in a low dose + 17 β -E ₂ rats	146.3 $\pm$ 12.6 [#]	4.9 $\pm$ 0.4
OVX/cholecalciferol in middle doses + 17 β -E ₂ rats	137.8 $\pm$ 14.4 [#]	5.3 $\pm$ 0.6
OVX/cholecalciferol in a high dose + 17 β -E ₂ rats	223.7 $\pm$ 12.6* [#]	13.5 $\pm$ 0.8* [#] ^{##}

* $p < 0.05$ as compared to the control group of sham-operated rats, [#] $p < 0.05$ as compared to the OVX rats treated with solvent, ^{##} $p < 0.05$ as compared to the OVX rats treated with 17 β -estradiol. Each group comprised a minimum of 8 rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. 17 β -Estradiol (17 β -E₂) was given at 0.5 μ g/rat, s.c., once daily, for 14 days. The obtained results show the mean $\pm$ S.E.M.

predict anxiolytic- or anxiogenic-like effects of drugs in rodents.^{36,39} Diazepam was applied as well-known anxiolytic positive control drug.⁴⁴ The results of the present study clearly demonstrated that diazepam is able to produce anxiolytic effect in intact/solvent and OVX/solvent rats. The anxiolytic-like effect of diazepam in intact/solvent and OVX/solvent rats is associated the reducing animal locomotion activity and grooming behavior as a result of sedative effect of this drug in the OFT.

Our results showed that cholecalciferol in high doses induced decrease of anxiety-like behavior of intact and OVX female rats in the EPM and LDT. Moreover, the anxiolytic-like effect of the high dose of cholecalciferol was similar to the effects observed after the reference anxiolytic drug diazepam. However, the current study has one

limitation. We did not measured the phase of ovary cycle in intact females. Further studies will focus on the assessment of the effect of cholecalciferol in different doses on anxiety-like behavior of female rats for all phases of ovary cycle. Furthermore, in OVX rats, there were marked anxiety-like behavior as assessed by EPM and LDT.

Although 17 β -E₂ supplementation resulted in a marked anxiolytic-like effect of OVX rats, the 17 β -E₂ administration alone or in combination with diazepam was not able to completely decrease anxiety-like behavior to the level of control intact animals. According to these results, we conclude that OVX rats display significant affected-related behavior, while 17 β -E₂ or diazepam administration to the OVX rats attenuates the estrogen deficiency-induced anxiety-like behavior to some extent. This

Table 4. Effects of chronic cholecalciferol administration on behavior of OVX females and OVX females treated with 17 β -estradiol in the open field test during 5 min

Groups	Crossing	Rearing	Grooming
Intact sham-operated rats + solvent (control/solvent rats)	56.3 $\pm$ 3.8	12.7 $\pm$ 0.8	3.6 $\pm$ 0.2
Intact sham-operated rats + diazepam (intact/diazepam rats)	28.9 $\pm$ 4.2*	5.4 $\pm$ 0.6*	1.4 $\pm$ 0.2*
Intact/cholecalciferol in a low dose rats	60.4 $\pm$ 2.4	11.2 $\pm$ 0.6	1.7 $\pm$ 0.2*
Intact/cholecalciferol in a middle dose rats	53.1 $\pm$ 4.8	14.2 $\pm$ 1.2	3.0 $\pm$ 0.2
Intact/cholecalciferol in a high dose rats	55.9 $\pm$ 3.6	12.1 $\pm$ 0.8	2.9 $\pm$ 0.2
OVX rats + solvent (OVX/solvent rats)	62.7 $\pm$ 4.2	13.4 $\pm$ 0.6	1.8 $\pm$ 0.2*
OVX rats + diazepam (OVX/diazepam rats)	36.3 $\pm$ 3.2* [#]	6.2 $\pm$ 0.8* [#]	1.6 $\pm$ 0.2* [#]
OVX rats + 17 β -E ₂ (OVX/17 β -E ₂ rats)	58.1 $\pm$ 3.4	12.0 $\pm$ 0.2	3.2 $\pm$ 0.4 [#]
OVX rats + 17 β -estradiol + diazepam (OVX/17 β -E ₂ /diazepam rats)	55.8 $\pm$ 4.5* [#]	11.2 $\pm$ 0.8* [#]	2.9 $\pm$ 0.5 [#]
OVX/cholecalciferol in a low dose rats	55.2 $\pm$ 2.8	12.2 $\pm$ 0.6	3.6 $\pm$ 0.2
OVX/cholecalciferol in a middle dose rats	53.7 $\pm$ 4.6	14.1 $\pm$ 1.8	3.6 $\pm$ 0.2
OVX/cholecalciferol in a high dose rats	66.9 $\pm$ 3.4	11.7 $\pm$ 0.4	5.9 $\pm$ 0.2* [#]
OVX/cholecalciferol in a low dose + 17 β -E ₂ rats	56.3 $\pm$ 3.6	10.9 $\pm$ 0.6	4.0 $\pm$ 0.6
OVX/cholecalciferol in a middle dose + 17 β -E ₂ rats	57.8 $\pm$ 3.4	15.3 $\pm$ 1.6	3.9 $\pm$ 0.2
OVX/cholecalciferol in a high dose + 17 β -E ₂ rats	64.7 $\pm$ 5.6	14.5 $\pm$ 0.8	7.6 $\pm$ 0.8* [#] ^{##}

*p < 0.05 as compared to the control group of sham-operated rats, [#]p < 0.05 as compared to the OVX rats treated with solvent, ^{##}p < 0.05 as compared to the OVX rats treated with 17 β -estradiol. Each group comprised a minimum of 8 rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. 17 β -Estradiol (17 β -E₂) was given at 0.5 μ g/rat, s.c., once daily, for 14 days. The obtained results show the mean $\pm$ S.E.M.

confirms the results of preclinical and clinical studies showing that 17 β -E₂ and diazepam administration can have anxiolytic-like effect in females.¹³⁻¹⁵

We found that treatment with cholecalciferol in high doses (5.0 mg/kg/day, s.c.) per se has a significant anxiolytic-like effect and is completely able to remove the anxiety-like profile in the OVX rats. Interestingly, administration of cholecalciferol in a high dose (5.0 mg/kg/day, s.c.) in combination with 17 β -E₂ in the OVX rats potentiated the anxiolytic-like effects of both preparations. On the other hand, cholecalciferol in a high dose (5.0 mg/kg/day, s.c.) alone or in combination with 17 β -E₂, significantly increased grooming events and so significantly reversed the anxiety-like profile in the OVX rats in the OFT. However, the results from OVX/17 β -E₂ rats and from the OVX/cholecalciferol in high dose (5.0 mg/kg/day, s.c.)+17 β -E₂ rats indicate that

cholecalciferol in a high dose (5.0 mg/kg/day, s.c.) affects anxiety-related processes rather than motor function. It should be emphasized that combinations of those substances, administered according to the same experimental schedule, did not increase locomotor activity. Thus, anxiolytic-like effect of cholecalciferol in high doses (5.0 mg/kg/day, s.c.) in the OVX rats was not induced by changes in motor function, because the general locomotor activity of these rats activity was not altered, in contrast to the effects observed after the reference anxiolytic drug diazepam in the OFT.

The OFT results in this study suggested that cholecalciferol in a high dose (5.0 mg/kg/day, s.c.) given alone or together with 17 β -E₂ to the OVX rats resulted in complete anxiolytic-like effects. Also, the results in the present study indicated that administration of cholecalciferol in high dose (5.0

Table 5. Effects of chronic cholecalciferol administration on monoamines and their metabolites levels (ng/mg wet tissue) of OVX females and OVX females treated with 17 β -estradiol detecting by HPLC in the hippocampus

Groups	DA	DOPAC	HVA	NA	3,4-MHPG	5-HT	5-HIAA
Intact sham-operated rats + solvent (Control/solvent rats)	0.28 $\pm$ 0.002	0.029 $\pm$ 0.005	0.02 $\pm$ 0.004	0.62 $\pm$ 0.03	0.06 $\pm$ 0.003	0.99 $\pm$ 0.02	0.93 $\pm$ 0.03
Intact sham-operated rats + diazepam (Intact/diazepam rats)	0.30 $\pm$ 0.002	0.028 $\pm$ 0.004	0.02 $\pm$ 0.003	0.60 $\pm$ 0.02	0.07 $\pm$ 0.004	0.98 $\pm$ 0.02	0.95 $\pm$ 0.08
Intact/cholecalciferol in a low dose rats	0.32 $\pm$ 0.06	0.036 $\pm$ 0.007	0.018 $\pm$ 0.005	0.59 $\pm$ 0.05	0.08 $\pm$ 0.002	1.00 $\pm$ 0.03	1.03 $\pm$ 0.08
Intact/cholecalciferol in a middle dose rats	0.26 $\pm$ 0.05	0.027 $\pm$ 0.006	0.021 $\pm$ 0.006	0.60 $\pm$ 0.04	0.09 $\pm$ 0.004	1.1 $\pm$ 0.04	0.89 $\pm$ 0.06
Intact/cholecalciferol in a high dose rats	0.24 $\pm$ 0.04*	0.03 $\pm$ 0.004	0.022 $\pm$ 0.004	0.61 $\pm$ 0.03	0.08 $\pm$ 0.002	1.05 $\pm$ 0.04	1.06 $\pm$ 0.05
OVX rats + solvent (OVX/solvent rats)	0.062 $\pm$ 0.002*	0.014 $\pm$ 0.002*	0.002 $\pm$ 0.0002*	0.22 $\pm$ 0.06*	0.076 $\pm$ 0.02	0.32 $\pm$ 0.06*	0.6 $\pm$ 0.02*
OVX rats + diazepam (OVX/diazepam rats)	0.058 $\pm$ 0.003*	0.013 $\pm$ 0.002*	0.002 $\pm$ 0.0007*	0.20 $\pm$ 0.05*	0.079 $\pm$ 0.04	0.30 $\pm$ 0.06*	0.55 $\pm$ 0.07*
OVX rats + 17 β -E ₂ (OVX/17 β -E ₂ rats)	0.12 $\pm$ 0.004*#	0.032 $\pm$ 0.002#	0.001 $\pm$ 0.0002*#	0.43 $\pm$ 0.06*#	0.02 $\pm$ 0.002*#	0.64 $\pm$ 0.06*#	2.0 $\pm$ 0.6*#
OVX rats + 17 β -estradiol + diazepam (OVX/17 β -E ₂ /diazepam rats)	0.11 $\pm$ 0.004*#	0.029 $\pm$ 0.003#	0.0011 $\pm$ 0.0006*#	0.45 $\pm$ 0.02*#	0.02 $\pm$ 0.002*#	0.7 $\pm$ 0.05*#	2.3 $\pm$ 0.6*#
OVX/cholecalciferol in a low dose rats	0.060 $\pm$ 0.02*#	0.015 $\pm$ 0.002*	0.0011 $\pm$ 0.0002*	0.25 $\pm$ 0.08*	0.082 $\pm$ 0.06*	0.30 $\pm$ 0.07*	0.65 $\pm$ 0.07*
OVX/cholecalciferol in a middle dose rats	0.066 $\pm$ 0.03*#	0.012 $\pm$ 0.002*	0.0012 $\pm$ 0.0002*	0.20 $\pm$ 0.04*	0.072 $\pm$ 0.09*	0.29 $\pm$ 0.05*	0.58 $\pm$ 0.03*

OVX/cholecalciferol in a high dose rats	0.34 ± 0.03 [#]	0.031 ± 0.002 [#]	0.016 ± 0.002 [#]	0.25 ± 0.07 [*]	0.077 ± 0.002	1.2 ± 0.2 [#]	0.22 ± 0.06 ^{*#}
OVX/cholecalciferol in a low dose + 17β-E ₂ rats	0.10 ± 0.006 ^{*#}	0.030 ± 0.002 ^{*#}	0.001 ± 0.0002 ^{*#}	0.48 ± 0.05 ^{*#}	0.018 ± 0.004 ^{*#}	0.71 ± 0.08 ^{*#}	2.1 ± 0.4 ^{*#}
OVX/cholecalciferol in a middle dose + 17β-E ₂ rats	0.13 ± 0.005 ^{*#}	0.029 ± 0.002 ^{*#}	0.001 ± 0.0002 ^{*#}	0.51 ± 0.03 ^{*#}	0.031 ± 0.002 ^{*#}	0.60 ± 0.06 [#]	2.0 ± 0.2 ^{*#}
OVX/cholecalciferol in a high dose + 17β-E ₂ rats	0.89 ± 0.02 ^{*#} ##	0.082 ± 0.02 ^{*#} ##	0.058 ± 0.006 [*] ###	0.46 ± 0.08 ^{*#}	0.024 ± 0.006 ^{*#}	2.4 ± 0.2 ^{*#} ###	0.27 ± 0.002 [*] ###

*- $P < 0.05$ as compared to the control group of sham-operated rats, [#]- $P < 0.05$ as compared to the OVX rats treated with solvent, ^{##}- $P < 0.05$ as compared to the OVX rats treated with 17β-estradiol. Each group comprised a minimum of 8 rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. 17β-Estradiol (17β-E₂) was given at 0.5 μg/rat, s.c., once daily, for 14 days. The obtained results show the mean ± S.E.M.

mg/kg/day, s.c.) in combination with 17β-E₂ could reverse the effect of the ovariectomy on grooming behavior. In the present study, only one concentration of 17β-E₂ was used because this concentration of 17β-E₂ had shown anxiolytic-like effects in OVX rats.^{14,15} Different dosages and duration of 17β-E₂ treatment should be tested in the future study.

Anxiety-related are the most common neuropsychiatric disorders in female patients.^{5,6,8} It has been postulated that the alterations of neurotransmission in the key brain areas such as the prefrontal cortex and hippocampus play a pivotal role in the progression of several neuropsychiatric diseases including anxiety, and the beneficial effects of VD in these brain-related disorders is, at least partially, via its modulating effect on neurotransmission.^{18,45} The hippocampus, with connections to precuneus, posterior cingulate cortex, lateral prefrontal cortex, and lateral temporoparietal cortex, has a key role in the default mode network, a network that supports numerous pathophysiological behavioral processes.^{46,47} Such deterioration of default mode network connectivity is accompanied by the emergence of cognitive symptoms including memory impairment and different neuropsychiatric disorders including anxiety.⁴⁷ Changes in the rat hippocampus monoamines or their metabolites were also performed to explain the observed pharmacological effects of cholecalciferol. HPLC data showed that cholecalciferol in a high dose treatment per se significantly increased

and completely restored 5-HT and DA levels, as well as decreased 5-HIAA levels in the hippocampus of OVX rats. Furthermore, administration of cholecalciferol in high doses in combination with 17β-E₂ in the OVX rats potentiated the 5-HT and DA levels. These data are in line with the similar effects of cholecalciferol in high dose treatment on the anxiety-related profile in the OVX rats. However, it should be noted that cholecalciferol in high doses (5.0 mg/kg/day, s.c.) treatment alone failed to alter 3,4-MHPG/NA ratio in the hippocampus of OVX rats, which is a key monoamine playing an important role in the mechanisms of anxiety-like behavior. Only co-administration of cholecalciferol in high doses with 17β-E₂, like 17β-E₂ alone, reduces 3,4-MHPG/NA ratio in the hippocampus.

There are some explanations for the anxiolytic-like effects of cholecalciferol in a high dose (in the OVX rats obtained in our present study using EPM and LDT as paradigms for assessment of anxiety-like behavior. The role of 5-HT in anxiety is now well established and it has been conclusively shown that increase in central serotonergic activity invariably leads to anxiety, whereas a decrease in the brain 5-HT activity results in anxiolysis.⁴⁸ 5-HT is suggested to regulate aspects of the development and the plasticity of brain circuits. Serotonin (5-HT) and γ-aminobutyric acid (GABA) show a reciprocal modulatory function in the hippocampus. This region is rich in both 5-HT and GABA terminals

Table 6. Effects of chronic cholecalciferol administration on monoamines/their metabolites ratio (ng/mg wet tissue) of OVX females and OVX females treated with 17 β -estradiol detecting by HPLC in the hippocampus

Groups	DOPAC/DA	HVA/DA	3,4-MHPG/NA	5-HIAA/5-HT
Intact sham-operated rats + solvent (Control/solvent rats)	0.103 $\pm$ 0.004	0.071 $\pm$ 0.002	0.097 $\pm$ 0.004	0.94 $\pm$ 0.02
Intact sham-operated rats + diazepam (Intact/diazepam rats)	0.093 $\pm$ 0.006	0.067 $\pm$ 0.004	0.11 $\pm$ 0.008	0.97 $\pm$ 0.04
Intact/cholecalciferol in a low dose rats	0.11 $\pm$ 0.004	0.056 $\pm$ 0.002	0.14 $\pm$ 0.04	1.00 $\pm$ 0.02
Intact/cholecalciferol in a middle dose rats	0.10 $\pm$ 0.003	0.08 $\pm$ 0.004	0.15 $\pm$ 0.02	0.81 $\pm$ 0.02
Intact/cholecalciferol in a high dose rats	0.125 $\pm$ 0.002	0.091 $\pm$ 0.06	0.13 $\pm$ 0.02	1.01 $\pm$ 0.07
OVX rats + solvent (OVX/solvent rats)	0.23 $\pm$ 0.03*	0.030 $\pm$ 0.002*	0.35 $\pm$ 0.02*	1.88 $\pm$ 0.2*
OVX rats + diazepam (OVX/diazepam rats)	0.22 $\pm$ 0.05*	0.034 $\pm$ 0.007*	0.39 $\pm$ 0.02*	1.83 $\pm$ 0.2*
OVX rats + 17 β -E ₂ (OVX/17 β -E ₂ rats)	0.27 $\pm$ 0.05*	0.0083 $\pm$ 0.002 [#]	0.046 $\pm$ 0.002* [#]	3.1 $\pm$ 0.2* [#]
OVX rats + 17 β -E ₂ + (OVX/17 β -E ₂ rats/diazepam)	0.26 $\pm$ 0.008*	0.01 $\pm$ 0.007 [#]	0.044 $\pm$ 0.002* [#]	3.28 $\pm$ 0.5* [#]
OVX/cholecalciferol in a low dose rats	0.25 $\pm$ 0.06*	0.018 $\pm$ 0.002 [#]	0.328 $\pm$ 0.02*	2.1 $\pm$ 0.2*
OVX/cholecalciferol in a middle dose rats	0.18 $\pm$ 0.04*	0.018 $\pm$ 0.002 [#]	0.36 $\pm$ 0.02*	2.0 $\pm$ 0.2*
OVX/cholecalciferol in a high dose rats	0.09 $\pm$ 0.002*	0.047 $\pm$ 0.003* [#]	0.31 $\pm$ 0.04*	0.18 $\pm$ 0.8* [#]
OVX/cholecalciferol in a low dose + 17 β -E ₂ rats	0.30 $\pm$ 0.05*	0.010 $\pm$ 0.004 [#]	0.038 $\pm$ 0.003* [#]	2.96 $\pm$ 0.4* [#]
OVX/cholecalciferol in a middle dose + 17 β -E ₂ rats	0.22 $\pm$ 0.08*	0.0077 $\pm$ 0.002 [#]	0.06 $\pm$ 0.02* [#]	3.3 $\pm$ 0.7* [#]
OVX/cholecalciferol in a high dose + 17 β -E ₂ rats	0.09 $\pm$ 0.004 [#] ##	0.065 $\pm$ 0.002 [#] ##	0.05 $\pm$ 0.006* [#]	0.11 $\pm$ 0.02* [#] ##

* - $P < 0.05$ as compared to the control group of sham-operated rats, [#] - $P < 0.05$ as compared to the OVX rats treated with solvent, ## - $P < 0.05$ as compared to the OVX rats treated with 17 β -estradiol. Each group comprised a minimum of 8 rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. 17 β -Estradiol (17 β -E₂) was given at 0.5 μ g/rat, s.c., once daily, for 14 days. The obtained results show the mean $\pm$ S.E.M.

and receptors.^{30,49} Projections from serotonergic neurons of the raphe nuclei terminate on GABAergic hippocampal interneurons increasing or decreasing their activity via serotonergic receptors.^{31,50} In our study of cholecalciferol effects in hippocampus may be useful to study the respective contribution direct of serotonin and/or indirect of GABA in the treatment of CNS diseases.

DA and NE are the most abundant excitatory monoamine neurotransmitters, widely distributed in the mammalian brain, including the hippocampus.⁵¹ There are some diseases associated with changes in these neurotransmitters levels. A role for the disrupted dopaminergic transmission in the etiology of chronic neurodegenerative diseases has been postulated.^{52,53} The neurochemical effects induced by cholecalciferol can explain its anxiolytic-like action in the OVX rats, particularly the induced decrease in 5-HIAA/5-HT ratio, as indicated by a decrease in 5-HIAA levels and an increase in 5-HT levels. Simultaneously, the increase in DOPAC/DA and HVA/DA ratios, as evidenced by the induced increase in the levels of DA and its metabolites, HVA, and DOPAC, can also contribute to the observed anxiolytic-like effects of cholecalciferol in high dose administration in the OVX and OVX/17 β -E₂ rats.

Some studies have demonstrated that VD has been shown to regulate the synthesis of a variety of neurotransmitters. Animal studies suggest that calcitriol can increase the expression of genes encoding for tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, and provide significant neuroprotection against the dopaminergic toxins by upregulating glial derived neurotrophic factor (GDNF).⁵⁴⁻⁵⁶ VD has also been found to affect the expression of genes associated with GABAergic neurotransmission⁴⁵ and to stimulate the expression of tyrosine hydroxylase (TH), responsible for catecholamine biosynthesis^{57,58}. Additionally, a recent study identified the presence of VD receptors on tryptophan hydroxylase (TPH) genes (which is considered as the rate-limiting step in the synthesis of the serotonin).⁵⁹ Altered GABA status has been found in the brain tissues of rodents fed with a VD deficient diet^{60,61}, and a significant reduction of glutamate decarboxylase (GAD) 67 and GAD65 protein levels has been observed in adult VD deficient mice⁵⁹.

DA has a modulatory effect on the dopaminergic system, in which increase of DA in the hippocampus is correlated with decreased glutamate levels. DA modulates fast excitatory synaptic transmission in

several brain regions. The results also suggest that the observed increase in DA level was not produced by the activation of the sparse dopaminergic projection from ventral tegmental area to the hippocampus^{60,61}, this increase was probably produced by inhibition of the glutamatergic system by cholecalciferol. In our study, the possible cholecalciferol effects in hippocampus may be useful to study the respective contribution direct of serotonin and/or indirect of GABA in the treatment of psychiatric diseases. We conclude that the modification of 5-HT and DA levels can be produced by increasing their synthesis and/or release; by reduction of their metabolism rate; and/or by decreasing its reuptake brain.

Thus, these findings demonstrate that chronic cholecalciferol administration may have beneficial effects on serotonergic and dopaminergic neurotransmitter systems. Based on our results, it may be suggested that cholecalciferol helps to provide some protection against ovariectomy-induced anxiety-like behavior by altering monoamine levels in the hippocampus. Further studies are needed to be done to understand the detailed mechanism of anxiolytic-like effect of cholecalciferol.

CONCLUSION

The results of the present preclinical study indicates that the effect of chronic cholecalciferol in a high dose treatment (5.0 mg/kg/day, s.c.) on anxiety-related behavior after ovariectomy-induced impairment can be explained by its direct and/or indirect dual action on emotional functions of the brain and modifications of monoamines and its metabolites levels. The data also indicate that the combination of cholecalciferol in high doses (5.0 mg/kg/day, s.c.) and 17 β -E₂ is more effective than 17 β -E₂ alone in OVX rats inducing a more profound anxiolytic-like effect in the EPM and the LDT. In summary, it can be suggested that the positive effect of chronic cholecalciferol in a high dose treatment (5.0 mg/kg/day, s.c.) on anxiety-related brain function is associated with its mutual and complex action on the monoaminergic systems, mainly the dopaminergic and serotonergic systems. Furthermore, this is the first study to show the beneficial effect of chronic cholecalciferol in high dose administration on anxiety-related states induced by ovariectomy in female rats with low estrogen levels. This work promotes more effective creating of the novel therapeutic targets and strategies for anxiety treatment in subjects with estrogen imbalance.

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Модуляция эффектов лечения холекальциферолом на тревожное поведение, вызванное дефицитом эстрогена у взрослых женских особей крыс

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Введение: Витамин Д может являться одним из возможных веществ, которые могут применяться в качестве дополнительной добавки в лечении тревожных расстройств женщин с эстрогенным дисбалансом.

Материалы и методы: Следовательно, целью настоящей работы являлось исследование эффектов воздействия постоянного применения холекальциферола (1.0, 2.5 или 5.0 мг/кг/день, с.к.) на тревожное поведение и уровни моноаминов в гиппокампе крыс после овариэктомии у женских особей. Холекальциферол был применен на овариэктомизированных (OVX) крысах и OVX крысах, получавших 17β-эстрадиол (17β-E2, 0.5 μg/крыса, с.к.). Тревожное поведение было исследовано с применением теста приподнятого крестообразного лабиринта (elevated plus maze - EPM) и теста перехода в малый отсек (light-dark tests - LDT), двигательная активность и гигиенические процедуры были исследованы с применением теста открытого поля (open-field test - OFT).

Результаты: Холекальциферол в высоких дозах самостоятельно или в комбинации с 17β-E2 вызывает подобные анксиолитическим эффекты у OVX крыс и OVX крыс, получавших 17β-E2, которые установлены при EPM тесте и LDT тесте и повышенную активность гигиенических процедур при OFT тесте. Также было установлено повышение уровней допамина и 5-HT в отличие от понижения обратного поглощения 5-HT в гиппокампе в данных группах OVX крыс.

Заключение: Наши данные показывают, что холекальциферол в высоких дозах обладает выраженным анксиолитическим эффектом ввиду повышения уровней моноаминов при экспериментальной модели крысы с эстрогенным дефицитом.

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Использованные сокращения в данной статье:

CNS: центральная нервная система; DA: допамин; DOPAC: 3,4-дигидроксифенилуксусная кислота; EPM: тест приподнятого крестообразного лабиринта; HPG система: гипоталамо-гипофизарно-гонадная система; HPLC: жидкостная хроматография под высоким давлением; LDT: тест перехода в малый отсек; MHPG: 3-метокси-4-гидроксифенил гликоля; NE: норадреналин; OFT: тест открытого поля; OVX: овариэктомизированный; VD: витамин D; 17 β -E2: 17 β -эстрадиол; 5-HIAA: 5-гидроксииндолуксусная кислота; 5-HT: 5-гидрокситриптамин, серотонин.