OM&P

Chronic Vitamin D3 Hormone Administration Reverses Affective-Related Profile in the Adult Female Rats after Long-Term Ovariectomy

Julia Fedotova [1,2]*

[1] Laboratory of Neuroendocrinology, I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences, 6 Emb. Makarova, St. Petersburg 199034, Russia;

[2] Department of Chemistry and Molecular biology, ITMO University, 49 Kronverksky pr., St. Petersburg 197101, Russia.

* Corresponding e-mail: julia.fedotova@mail.ru

Abstract. The aim of the current study was to examine the effects of chronic cholecalciferol administration (1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days) on the anxiety-like and depression-like behaviors following long-term ovariectomy (12 weeks) in female rats. Cholecalciferol was administered to the ovariectomized (OVX) rats and OVX rats treated with 17β-estradiol after long-term absence of estrogen (17β-E2, 0.5 μg/rat, s.c., once daily, for 14 days). Anxiety-like behavior was assessed in the elevated plus maze (EPM), depression-like behavior was assessed in the forced swimming test (FST), locomotor and grooming activities were assessed in the open field test (OFT). The treatment with cholecalciferol (1.0 mg/kg/day, s.c.) in the OVX rats after long-term absence of estrogens induced antidepressant-like effect ($p<0.05$). Moreover, cholecalciferol in this dose plus 17β-E2 more markedly exhibited antidepressant-like effect in the OVX rats after long-term ovariectomy ($p<0.05$). The OVX rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg demonstrated a decrease of anxiety-like behavior in the EPM. The combination of cholecalciferol at doses of 1.0 and 2.5 mg/kg with a low dose of 17β-E2 more effectively decreases anxiety-like behavior in the OVX rats after long-term estrogen deficiency than17β-E2 alone. This work promotes more effective creation of novel therapeutic targets and strategies for affective-related disorders treatment in female subjects with long-term estrogen deficiency.

Introduction

Affective-related disorders are disabling conditions, which often leads to significant personal, societal, and economic costs (Soares, Maki, 2010; Soares, 2013). It is well-known that women are more vulnerable than men for development of mood disorders (Kronke et al., 2007; Martin-Merino et al., 2010). In fact, the onset or exacerbation of mood disturbances has been associated to menopause (Burger, 2008; Bromberg et al., 2013). Hormone replacement therapy (HRT) has been widely available and used to ameliorate physiological and behavioral alterations in the postmenopausal women (Vera, Rada, 2002). However, HRT accepted as the gold standard for estrogen replacement during menopause, alternative and additional treatments that are more effective are continuously being sought (Scheid et al., 2010). Considering the wide use of complementary and alternative medications such as vitamin supplements in menopausal patients and our insufficient knowledge about the interaction between hormone replacement therapy and vitamin supplements, investigating the subject from a preclinical experimental studies seems very beneficial (Peng et al., 2016).

Vitamine D (VD) can be one of such candidate substance as additional supplementation for treatment of affective-related disorders in women with an imbalance of estrogens. In addition to its classic role in bone metabolism, VD may also have many potential non-skeletal functions (Stewart et al., 2010; Kesby et al., 2011; Wrzosek et al., 2013). VD has important functions in the human brain and may play a role in affective-related disorders (Eyles et al., 2005; 2013). VDRs are present in multiple brain regions associated with depressive disorders, including the prefrontal cortex and hippocampus, and cells in many of these regions are capable of metabolizing 25-hydroxyvitamin D to the biologically active metabolite 1,25-dihydroxyvitamin D (Eyles et al., 2014). Animal studies have suggested that VD may increase the synthesis and/or metabolism of neurotransmitters, including serotonine, dopamine and norepinephrine (Wang et al., 2001; Patrick et al., 2014). Taking into account the potential therapeutic role of VD in mood disorders, we designed the present study to determine the therapeutic effects of VD as an adjunctive therapy alone or in a combination with low dose of 17β-estradiol on anxiety- and depression-like behaviors of female rats after long-term absence of estrogen. In rats, long-term absence of ovarian hormones induced by ovariectomy has been proposed as an early model of postmenopause (Bosee, Di Paolo, 1995). It was found that rats with 12-weeks postovariectomy showed greater parameters of anxiety- and depression-like states than rats with 3 weeks postovariectomy (Picazo et al., 2006; Estrada-Camarena et al., 2017). Thus, the rats with chronic absence of ovarian hormones induced by long-term ovariectomy might reflect mood disturbances typical of human menopause (Picazo et al., 2006). Thus, it is a great interest to evaluate the effects of repeated cholecalciferol administration on affective-related behavior in the adult female rats with long-term estrogen deficiency.

The aim of the present study was to determine if repeated systemic treatment with cholecalciferol affected anxiety- and depression-related behaviors in female rats after long-term ovariectomy. Moreover, it is interesting to clarify whether after repeated treatment of cholecalcifeol, its effects on affective-related behavior may be determined and depended from the hormonal state of female rats (low estrogen level or 17β-estradiol application). Therefore, another aim of this work was to investigate whether repeated combined treatment with cholecalciferol plus 17β-estradiol (17β-E2) could affect on anxiety- and depression-related behaviors more than 17β-E2 alone in female rats after long-term ovariectomy.

Materials and Methods

Animals

The study used 288 of the adult (3 months old) female Wistar line rats (purchased from «Biocollection of I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences», Koltushi, St. Petersburg, Russia) weighing 180-200 g at the start of the experiment. For at least a week prior to the experiment, the adult rats were housed six to a cage under standard environmental conditions: constant temperature of 23 ± 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 the I.P. Pavlov Institute of Physiology, approved by the Scientific Research Committee of the Institute (protocol 1095/1 from June 25, 2012). The rationale, design, and methods of this study were approved by the Ethical Committee for Animal Research, I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences.

Long-term ovariectomy

In rats, long-term absence of ovarian hormones during 12 weeks induced by ovariectomy has been proposed as an early model of postmenopause (Bosee, Di Paolo, 1995). In the present study, female rats for chronic experiment were subjected by long-term 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 oversee 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. Following ovariectomy, ovariectomized (OVX) females were placed in a community cage with free access to food and water. After the surgery and to assure the long-term absence of ovarian hormones, the rats were returned to the housing facilities for 12 weeks. After this time period, the rats were randomly assigned to each of the experimental groups accordingly to their age and subjected to solvent, cholecalciferol or 17β-E2 treatments.

Drugs

The estrogen, 17β-E2 (E-8875, Sigma Chemical Co) was dissolved in sterile sesame oil. Cholecalcirefol (C-9756, Sigma Chemical Co) was dissolved in 95% ethanol, aliquoted and stored at -800C. The stock of cholecalciferol was diluted in a sterile water, resulting in a solution of cholecalciferol with 2% ethanol. 17β-E2 was injected subcutaneously (s.c.) at a dose of 0.5 μg/rat. Ovariectomy markedly decreases estrogen level and 17β-E2 receptor activity in the different structures of the brain (Stanzione et al., 1984; Pick et al., 1995). In this connection, a low dose of 17β-E2 may play a trigger role in activation of 17β-E2 receptors at the hypoestrogenic syndrome (Picazo et al., 2006). Thus, we used a low dose of 17β-E2 in our present study. The low dose of 17β-E2 (0.5 μg/rat, s.c.) was chosen from the studies performed by Estrada-Camarena and co-workers (Estrada-Camarena et al., 2003, 2004). Cholecalcirefol was injected subcutaneously (s.c.) at three different doses (1.0, 2.5 or 5.0 mg/kg/day). Three doses of cholecalciferol were chosen from the behavioral study performed by Idrus and co-workers (Idrus et al., 2013). All solutions were freshly prepared before each experimental series. All preparations were administered in a volume of 0.1 ml. Following 12 weeks after ovariectomy, cholecalciferol, 17β-E2 and oil solvent were injected once daily for 14 days. The adult OVX females were 6.5 months old at the onset of pharmacological treatments.

Experimental groups

In our previous studies (data are not shown), we did not find any significant differences between control intact (sham-operated) rats treated with oil solvent and intact

 doi:10.20388/omp2017.003.0051

(sham-operated) females treated with sterile water with 2% ethanol as solvent for cholecalciferol in behavioral tests for measurement of anxiety- and depression-like states (data are not shown). Since, we did not found any differences between control groups of intact females with oil solvent and solvent for cholecalciferol, we used only one control intact (sham-operated) group with oil solvent.

Twelve weeks after ovariectomy, the adult OVX female rats were randomly assigned to each of the experimental groups and subjected to the different treatments. All female OVX and intact rats were divided into 12 groups (n=8 per group) for each behavioral tests. The first group consisted of intact (sham-operated) female rats (control) daily treated with oil solvent (control + solvent). The three other groups were of intact (sham-operated) female rats which received cholecalciferol at a daily dose of 1.0 mg/kg, s.c. (intact rats + cholecalciferol 1.0 mg/kg), cholecalciferol at a daily dose of 2.5 mg/kg, s.c. (intact rats + cholecalciferol 2.5 mg/kg) or cholecalciferol at a daily dose of 5.0 mg/kg, s.c. (intact rats + cholecalciferol 5.0 mg/kg). The next two groups were of OVX female rats received the oil solvent daily (OVX + solvent) and OVX rats treated with 17β-E2 at a daily dose of 0.5 μg/rat, s.c. (OVX + 17β-E2). The other groups consisted of the OVX female rats treated with cholecalciferol at a dose of 1.0 mg/kg (OVX rats + cholecalciferol 1.0 mg/kg), OVX female rats treated with cholecalciferol at a dose of 2.5 mg/kg (OVX rats + cholecalciferol 2.5 mg/kg), OVX female rats treated with cholecalciferol at a dose of 5.0 mg/kg (OVX rats + cholecalciferol 5.0 mg/kg), OVX female rats treated with cholecalciferol at a dose of 1.0 mg/kg plus 17β-E2 (OVX rats + cholecalciferol 1.0 mg/kg + 17β-E2), OVX female rats treated with cholecalciferol at dose of 2.5 mg/kg plus 17β-E2 (OVX rats + cholecalciferol 2.5 mg/kg + 17β-E2), and OVX female rats treated with cholecalciferol at a dose of 5.0 mg/kg plus 17β-E2 (OVX rats + cholecalciferol 5.0 mg/kg + 17β-E2).

To summarize the treatment workflow, after induction of the experimental model of estrogen deficiency, the OVX rats were left to recover for 12 weeks. After that time, the adult OVX female rats began daily injections for 14 days with either cholecalciferol, 17β-E2 or oil solvent. One hour after the last injection, testing in the forced swimming test (FST) and the open field test (OFT) was carried out as described below. During all behavioral tests, the experimental groups of the adult OVX rats were also treated with cholecalciferol, 17β-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 before each test. Any environmental or physical stress were 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%).

Forced swimming test

To investigate the changes in depression-like behavior, all experimental groups of OVX female rats with long-term absence of estrogen were subjected to an adapted version of the forced swimming test (FST) (Porsolt et al., 1978). A cylindrical container (height 60 cm; diameter 20 cm) was filled with 23 ± 2 0C water up to a level of 30 cm. In the first session (day 1, pretest), rats were placed in water for a 15 min assessment. Then, they were removed from the water and allowed to dry in a heated room before being returned to their home cages. Twenty-four hours later (day 2, test), rats were put back into the cylinder for 5 min and latency and duration of immobility behavior (floating in the water with only movements necessary to keep the head above water) were measured by an observer blind to the rat treatment. Since pharmacologically psychotropic drugs affect different patterns of active behavior in the FST, swimming behavior (active swimming movements around cylinder) and climbing behavior (active movements with forepaws usually directed towards the walls) were also scored. A video camera was installed above the cage to record the activity of the rats. Two independent observers measured the behavioral variables.

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 with long-term absence of estrogen were subjected to the elevated plus maze test (EPM) (Pellow, File, 1986). EPM is a widely used test of anxiety-like behavior and was used to assess an anxiety-like behavioral responses (Pellow, File, 1986). This test is sensitive to putative anxiogenic-like and anxiolytic-like drugs (Menzaghi et al., 1994). 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 in the size of 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 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.

Open field test

To investigate the changes in spontaneous locomotor activity, grooming, and rearing, all experimental groups of female rats were submitted to a 5-min period to the open field test (OFT) as described previously (Fedotova et al., 2012). 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.

Statistical analysis

All values were expressed as mean ± S.E.M. Comparisons between values were performed using two-way ANOVA test 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 software.

Results

Effects of cholecalciferol administration on depression-like behavior of OVX rats following long-term estrogen deficiency in the forced swimming test

A two-way ANOVA revealed significant differences in the immobility time between hormone conditions ([F(5,26) = 7.14, P<0.0001]), between drug treatments [F(5,26) = 11.09, P<0.05]), and an interaction between hormone condition and treatments ([F(5,26) = 12.56, P<0.05]) in the OVX rats with long-term estrogen deficiency-induced depression. The post-hoc test revealed differences among the groups for depression-like behavior in the FST ($p < 0.05$).

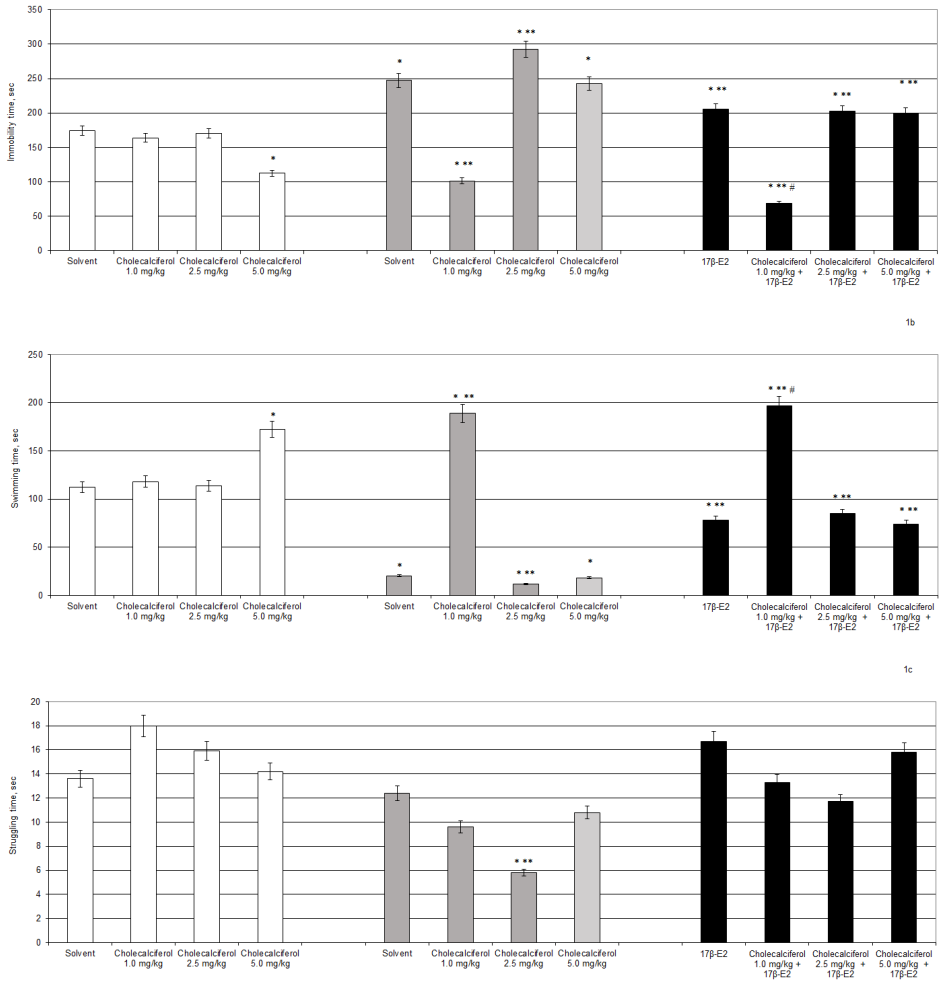

Figure 1. *Effects of cholecalciferol administration on depression-like behavior of ovariectomized (OVX) rats following long-term estrogen deficiency in the forced swimming test.*
(a) – immobility time, sec; (b) – swimming time, sec; (c) – struggling time, sec.
** - P < 0.05 as compared to the control 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, during 14 days.*

 doi:10.20388/omp2017.003.0051

The intact rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg showed no changes of the immobility time in the FST as compared to the control rats (Figure 1a, $p > 0.05$). The intact rats treated with cholecalciferol at a dose of 5.0 mg/kg showed a significant decrease in the immobility time as compared to the control rats (Figure 1a, $p < 0.05$).

Long-term ovariectomy in female rats resulted in a significant increase of the immobility time in the FST as compared to the control females (Figure 1a, $p < 0.05$). The 17β-E2 supplementation (0.5 μg/kg, SC) caused a decrease in the immobility time in the OVX rats as compared to the OVX rats administered with solvent (Figure 1a, $p < 0.05$). Although, the value of this parameter in the OVX/17β-E2 females were lower than that of the OVX treated with solvent rats, it did not reach the value of control rats.

The OVX rats treated with cholecalciferol at a dose of 1.0 mg/kg showed a significant decrease the immobility time as compared to the OVX and intact rats given with solvent (Figure 1a, $p < 0.05$). On the contrary, the OVX rats treated with cholecalciferol at a dose of 2.5 mg/kg showed an increase in the immobility time as compared to the OVX and intact rats given with solvent (Figure 1a, $p < 0.05$). Cholecalciferol treatment (5.0 mg/kg) failed to modify immobility time in the FST as compared to the OVX/solvent rats (Figure 1a, $p > 0.05$).

Administration of cholecalciferol at a dose of 1.0 mg/kg in combination with 17β-E2 more significantly decreased the immobility time for the OVX rats as compared to the intact, OVX females treated with oil solvent or 17β-E2 (Figure 1a, $p<0.05$). Combined administration of cholecalciferol at doses of 2.5 and 5.0 mg/kg and 17β-E2 in the OVX rats significantly decreased the immobility time, likely administration of 17β-E2 alone as compared to the intact and OVX rats given with solvent (Figure 1a, $p < 0.05$).

The significant differences in the swimming time were found between hormone conditions ($[F(5,26) = 5.96$, $P<0.01]$), between drug treatments $[F(5,26) = 9.14, P<0.05]$), and an interaction between hormone condition and treatments ($[F(5,26) = 15.22, P<0.01]$) in the OVX rats with long-term estrogen deficiency-induced depression. The post-hoc test revealed differences among the groups for swimming behavior in the FST ($p<0.05$).

The intact rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg failed to demonstrate any modifications of the swimming time in the FST as compared to the control rats (Figure 1b, $p > 0.05$). The intact rats treated with cholecalciferol at a dose of 5.0 mg/kg showed a significant increase in the swimming time as compared to the control/solvent rats (Figure 1b, $p < 0.05$).

The OVX/solvent rats showed a more significant decrease of the swimming time as compared to the control/solvent rats ($p<0.05$, Figure1b). Administration of 17β-E2 significantly increased the swimming time when OVX/17β-E2 rats were compared to the OVX rats given solvent ($p<0.05$, Figure 1b). However, the value of swimming time in the OVX treated with 17β-E2 rats was significant decreased compared to the control intact rats.

The OVX rats treated with cholecalciferol at a dose of 1.0 mg/kg showed a significant increase the swimming time as compared to the OVX and intact rats given with solvent (Figure 1b, $p < 0.05$). The OVX rats administered with cholecalciferol at a dose of 2.5 mg/kg showed a decrease in the swimming time as compared to the OVX and intact rats given h solvent (Figure 1b, $p < 0.05$). Cholecalciferol treatment (5.0 mg/kg SC) failed to alter swimming time in the FST as compared to the OVX/solvent rats (Figure 1b, $p > 0.05$).

The combined administration of cholecalciferol at a dose of 1.0 mg/kg in combination with 17β-E2 more significantly increased the swimming time for the OVX rats as compared to the intact, OVX females treated with oil solvent or 17β-E2 (Figure 1b, $p<0.05$). Combined administration of cholecalciferol at doses of 2.5 and 5.0 mg/kg and 17β-E2 in the OVX rats significantly elevated the swimming time, likely administration of 17β-E2 alone as compared to the intact and OVX rats given with solvent (Figure 1b, $p < 0.05$).

The significant differences in the struggling time were found between hormone conditions ($[F(5,26) = 9.34$, $P<0.01]$), between drug treatments $[F(5,26) = 5.11, P<0.05]$), and an interaction between hormone condition and treatments ($[F(5,26) = 11.68, P<0.01]$) in the OVX rats with long-term estrogen deficiency-induced depression. The post-hoc test revealed differences among the groups for struggling behavior in the FST ($p<0.05$). The OVX rats treated with cholecalciferol at a dose of 2.5 mg/kg showed a decrease in the struggling time as compared to the OVX and intact rats given with solvent (Figure 1c, $p < 0.05$). However, there were no significant changes in the struggling behavior in the control rats and other experimental groups of rats ($p<0.05$, Figure 1c).

Effects of cholecalciferol administration on anxiety-like behavior of OVX rats following long-term estrogen deficiency in the elevated plus maze

A two-way ANOVA revealed significant differences in the time spent into the open arms between hormone conditions ($F(5,32) = 11.41$, $p < 0.0001$), between drug treatments ($F(5,32) = 15.07$, $p < 0.0001$), and an interaction between hormone condition and treatments ($F(5,32) = 3.01$, $p < 0.001$) in the OVX rats with long-term estrogen deficiency-induced anxiety. The post-hoc test revealed differences among the groups for anxiety-like behavior in the EPM ($p < 0.05$).

The intact rats treated with cholecalciferol at a doses of 1.0 mg/kg and 2.5 mg/kg showed no modification in the time spent in the open arms as compared to the control rats (Table 1, $p > 0.05$). The intact rats treated with cholecalciferol at a dose of 5.0 mg/kg showed a significant increase in the time spent in the open arms as compared to the control/solvent rats (Table 1, $p < 0.05$). Long-term ovariectomy in female rats resulted in a significant decrease of the time spent in the open arms as compared to the control females (Table 1, $p < 0.05$). The 17β-E2 supplementation (0.5 μg/kg, s.c.) caused an

increase in the time spent in the open arms in the OVX rats as compared to the OVX rats administered solvent (Table 1, $p < 0.05$). The OVX rats treated with cholecalciferol at a dose of 1.0 mg/kg and 2.5 mg/kg showed an increase in the time spent into the open arms in dose-dependent manner as compared to the OVX rats given with solvent (Table 1, $p < 0.05$). Cholecalciferol treatment (5.0 mg/kg) significantly decreased the time spent in the open arms in the OVX rats as compared to the OVX and control rats given solvent (Table 1, $p < 0.05$). Administration of cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg in combinationwith17β-E2 more significantly increased the time spent in the open arms for the OVX rats as compared to the OVX females treated with oil solvent or 17β-E2 (Table 1, $p<0.05$). The time spent in the open arms for OVX rats administered cholecalciferol at a dose of 5.0mg/kg in combination with 17β-E2 was significantly greater than that of the OVX rats give solvent, and did not reach the value of control sham-operated rats (Table 1, $p < 0.05$). Moreover, the values of time spent in the open arms of OVX rats administered with cholecalciferol at a dose of 5.0 mg/kg in combination with 17β-E2 were similar to the values for OVX rats treated with 17β-E2.

Similarly, significant differences in the number of entries into the open arms were found between hormone conditions ($F(5,32) = 3.96$, $p < 0.01$), between drug treatments ($F(5,32) = 9.20$, $p < 0.001$), 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 rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg showed no alteration in the number of entries into the open arms as compared to the control rats (Table 1, $p > 0.05$). The intact rats treated with cholecalciferol at a dose of 5.0 mg/kg showed a significant increase with respect to the number of entries into the open arms as compared to the control rats (Table 1, $p < 0.05$). Administration of 17β-E2 to the OVX rats increased the number of entries into the open arms as compared to the OVX rats treated with solvent (Figure 1b, $p < 0.05$). The number of entries into the open arms was significantly higher when the OVX rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg were compared to the OVX rats given with solvent (Table 1, $p < 0.05$). Cholecalciferol at dose of 5.0 mg/kg failed to change the number of entries into the open arms in the OVX rats as compared to the OVX solvent-receiving rats (Table 1, $p < 0.05$). Administration of doses of 1.0mg/kg and 2.5mg/kg in combination with 17β-E2 in the OVX rats more significantly increased the number of entries into the open arms as compared to the OVX rats treated with solvent or 17β-E2 (Table 1, $p < 0.05$). The number of entries into the open arms of OVX rats administered cholecalciferol at a dose of 5.0 mg/kg in combination with 17β-E2 was lower than that of the OVX rats given solvent, but this did not reach the value of control sham-operated rats (Table 1, $p < 0.05$). Furthermore, the value for the number of entries into the open arms of the OVX rats administered cholecalciferol at a dose of 5.0 mg/kg plus 17β-E2 was similar to the value for OVX rats treated with 17β-E2.

Table 1. Effects of chronic cholecalciferol administration on anxiety-like behavior of OVX females and OVX females treated with 17β-estradiol in the elevated plus maze.

Groups	Time spent into the open arms	The number of entries into the open arms
Control rats + solvent	77.8 ± 6.2	2.6 ± 0.4
Intact rats + cholecalciferol 1.0 mg/kg	71.7 ± 9.4	1.7 ± 0.2
Intact rats + cholecalciferol 2.5 mg/kg	82.4 ± 6.2	1.6 ± 0.4
Intact rats + cholecalciferol 5.0 mg/kg	111.3 ± 10.2*	4.5 ± 0.6*
OVX rats + solvent	39.0 ± 12.2*	0.8 ± 0.2*
OVX rats + 17β-E_2	59.2 ± 6.4*	0.6 ± 0.2*
OVX rats + cholecalciferol 1.0 mg/kg	58.1 ± 8.2* #	1.4 ± 0.2* #
OVX rats + cholecalciferol 2.5 mg/kg	57.8 ± 3.4* #	2.8 ± 0.2#
OVX rats + cholecalciferol 5.0 mg/kg	70.1 ± 5.6 #	2.5 ± 0.2#
OVX rats + cholecalciferol 1.0 mg/kg + 17β-E_2 rats	6.3 ± 0.2* #	1.0 ± 0.6* #
OVX rats + cholecalciferol 2.5 mg/kg + 17β-E_2 rats	86.2 ± 5.1# ##	3.9 ± 0.4# ##
OVX rats + cholecalciferol 5.0 mg/kg + 17β-E_2 rats	89.8 ± 2.7# ##	4.6 ± 0.6# ##
Control rats + solvent	63.4 ± 4.6* #	1.5 ± 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 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, during 14 days.*

 doi:10.20388/omp2017.003.0051

Effects of Cholecalciferol administration on behavioral alterations of OVX rats following long-term estrogen deficiency in the open field test

The two-way ANOVA revealed significant differences in the crossing, rearing and grooming behaviors between hormone conditions ($F(5,34) = 5.44$, $p<0.05$), ($F(5,34) = 9.40$, $p<0.01$), ($F(5,34) = 15.34$, $p<0.01$), between drug treatments ($F(5,34) = 15.4$, $p<0.001$), ($F(5,34) = 11.56$, $p<0.05$), ($F(3,34) = 11.86$, $p<0.05$), and an interaction between hormone condition and treatments ($F(5,34) = 3.79$, $p<0.01$), ($F(5,34) = 5.46$, $p<0.05$), ($F(5,34) = 4.8$, $p<0.05$), in the OVX rats with long-term estrogen deficiency. The post-hoc test revealed differences among the groups for behavior in the OFT ($p<0.05$).

The sham-operated female rats treated with cholecalciferol at a dose of 1.0 mg/kg showed a significant decrease of grooming as compared to the control rats (Table 2, $p < 0.05$). The post-hoc test failed to demonstrate any alterations in behavioral reactions in the intact rats treated with cholecalciferol at doses of 2.5 mg/kg and 5.0 mg/kg as compared to the control rats (Table 2, $p > 0.05$).

OVX rats given with solvent exhibited a significant decrease of grooming behavior as compared to the control rats (Table 2, $p < 0.05$). The 17β-E2 supplementation produced a significant increase in grooming reactions when these rats were compared to the OVX rats treated with solvent (Table 2, $p < 0.05$).

The post-hoc test failed to reveal any alterations of motor and rearing activities in the OVX rats treated with cholecalciferol in all tested doses as compared to the OVX rats (Table 2, $p < 0.05$). However, cholecalciferol administration in all doses (1.0, 2.5 and 5.0 mg/kg, s.c.) to the OVX rats resulted in a significant elevated frequency of grooming as compared to the OVX rats treated with solvent (Table 2, $p < 0.05$).

The combination of cholecalciferol at dose of 1.0 mg/kg and 2.5 mg/kg with 17β-E2 enhanced motor activity as compared to the intact control females, OVX rats treated with 17β-E2 or solvent. However treatment with cholecalciferol at a doses of 1.0 mg/kg and 2.5 mg/kg plus 17β-E2 failed to modify grooming behavior when these groups of rats were compared to the OVX rats given solvent (Table 2, $p<0.05$). The values of these parameters in above-mentioned groups of OVX rats treated with cholecalciferol at doses of 1.0 mg/kg and 2.5 mg/kg in a combination with 17β-E2 were lower than that in the control or OVX rats treated with only 17β-E2. The OVX rats received with cholecalciferol at a dose of 5.0 mg/kg with 17β-E2 demonstrated increase of grooming behavior as compared to the OVX rats given solvent or 17β-E2 (Table 2, $p< 0.05$). Cholecalciferol at a dose of 5.0 mg/kg in a combination with 17β-E2 in the OVX rats failed to induce any changes of locomotor activity when these rats were compared to the OVX rats treated with 17β-E2 (Table 2, $p > 0.05$).

Discussion

We investigated the effects of chronic cholecalciferol treatment in different doses (1.0, 2.5, 5.0 mg/kg, s.c.) for 14 days on anxiety- and depression-like behaviors in female rats with long-term estrogen deficiency for 12 weeks. The results of behavioral testing for the anxiety- and depression-related effects of cholecalciferol

Table 2. Effects of chronic cholecalciferol administration on behavior of OVX females and OVX females treated with 17β-estradiol after long-term absence of estrogen in the open filed test during 5 min

Groups	Crossing	Rearing	Grooming
Control rats + solvent	61.2 ± 3.6	13.3 ± 0.4	3.7 ± 0.2
Intact rats + cholecalciferol 1.0 mg/kg	60.3 ± 2.4	13.2 ± 2.8	1.7 ± 0.4*
Intact rats + cholecalciferol 2.5 mg/kg	63.4 ± 4.5	12.1 ± 2.2	3.0 ± 0.6
Intact rats + cholecalciferol 5.0 mg/kg	59.2 ± 3.6	11.1 ± 0.8	3.4 ± 0.2
OVX rats + solvent	66.4 ± 2.3	11.4 ± 1.8	1.4 ± 0.5*
OVX rats + 17β-E_2	57.5 ± 3.4	16.1 ± 0.5	3.0 ± 0.2**
OVX rats + cholecalciferol 1.0 mg/kg	78.2 ± 4.3	15.4 ± 1.6	3.8 ± 0.2**
OVX rats + cholecalciferol 2.5 mg/kg	74.1 ± 2.2	13.9 ± 1.8	3.3 ± 0.2**
OVX rats + cholecalciferol 5.0 mg/kg	76.3 ± 4.6	12.7 ± 1.2	4.2 ± 0.2**
OVX rats + cholecalciferol 1.0 mg/kg + 17β-E_2 rats	92.5 ± 3.9* ** #	14.1 ± 1.2	1.7 ± 0.4* #
OVX rats + cholecalciferol 2.5 mg/kg + 17β-E_2 rats	93.2 ± 3.3* ** #	12.3 ± 1.6	2.0 ± 0.8* #
OVX rats + cholecalciferol 5.0 mg/kg + 17β-E_2 rats	67.0 ± 4.5	13.1 ± 1.2	4.2 ± 0.3** #

**– p<0.05 as compared to the control group of sham-operated rats, ** – p < 0.05 as compared to the OVX rats after long-term absence of estrogen treated with solvent, # – p < 0.05 as compared to the OVX rats after long-term absence of estrogen treated with 17β-estradiol. Each group comprised a minimum of eight rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day, s.c., once daily, for 14 days. The administered dose of 17β-E2 was 0.5 μg/rat, s.c., once daily, for 14 days.*

were compared in both OVX rats and OVX female rats treated with 17β-E2 with long-term estrogen deficiency. Simultaneously, effects of cholecalciferol treatment in similar doses on anxiety- and depression-like behaviors were carried out in intact female rats. For this purpose, the elevated plus maze (EPM) and forced swimming test (FST) were performed in this study.

Our results showed that cholecalciferol only at a dose of 5.0mg/kg decreased anxiety- and depression-like behaviors of intact rats in the EPM and FST. The current study has some limitations. We did not measured the phase of ovary cycle in intact females. The next step will be assessment of cholecalciferol in different doses effects on depression-like behavior in female rats for all phases of ovary cycle. Moreover, the age influence assessment in ovary-intact female rats on the behavioral effects of cholecalciferol administered at different doses in the FST is also needed to be done.

Our results showed that in OVX rats following 12 weeks of postovariectomy period, there were marked anxiety- and depression-like behaviors as assessed by EPM and FST, respectively. Although 17β-E2 supplementation resulted in significant anxiolytic- and antidepressant-like effects of OVX rats with long-term absence of estrogen, the 17β-E2 administration was not able to completely diminish anxiety- and depression-like behaviors to the level of control intact animals. According to these results, we conclude that OVX rats following 12 weeks of post-OVX period display significant affected-related behavior, while 17β-E2 administration to the OVX rats attenuates the estrogen deficiency-induced affective-like behavior to some extent. The long-term effect of ovariectomy on anxiety- and depression-like behaviors in female rats were submitted in a standard behavioral tests (Okada et al., 1997; Fedotova et al., 2004; Marshall, 2011; Fedotova, 2013; Nelly et al., 2016; Estrada-Camarena et al., 2017).

In the FST, we found that cholecalciferol at dose of 1.0 mg/kg/day per se had a significant antidepressant-like effect in the OVX rats following long-term ovariectomy. On the contrary, cholecalciferol at dose of 2.5 mg/kg/day SC exhibited a prodepressant-like effect in the OVX rats with long-term absence of estrogen. Interestingly, cholecalciferol administered at a dose of 5.0 mg/kg SC failed to modify depression-like behavior in these OVX females. In the EPM, we found that cholecalciferol at doses of 1.0 and 2.5 mg/kg per se had a significant anxiolytic-like effect in the OVX rats with long-term absence of estrogen. Simultaneously, cholecalciferol treatment in all tested doses similarly increased grooming and did not change locomotor activity of the OVX rats after long-term ovariectomy, however, its effects on the manifestation for anxiety- and depression-like behaviors of these rats were completely different in the EPM and FST. These data suggested that the completely different effects of cholecalciferol application at doses of 1.0, 2.5 and 5.0 mg/kg (antidepressant-like effect and prodepressant-like effect) in the OVX rats with long-term absence of estrogen on depression-like behavior in the FST did not associated with its effects on behavioral reactions in the OFT. The obtained data generally confirmed the anxiolytic- and antidepressant-like activities of cholecalciferol in the OVX females with long-term absence of estrogen and indicates that the effects of cholecalciferol are specific, since any alterations in motor activity were not involved in the action in the EPM and FST tests.

Administration of cholecalciferol at dose of 1.0 mg/kg/day in a combination with low dose of 17β-E2 in the OVX rats after long-term absence of estrogen exhibited synergic action and potentiated the antidepressant-like effects of both preparations in the FST. Moreover, administration of cholecalciferol at doses of 1.0 and 2.5 mg/kg in a combination with 17β-E2 to the OVX rats potentiated the anxiolytic-like effects of both preparations. Nevertheless, it should be emphasized that combinations of those substances, administered according to the same experimental schedule, increase locomotor activity. The results from the OVX/cholecalciferol 1.0 mg/kg +17β-E2 rats indicate that cholecalciferol in that dose affect both motor function and depression-related processes. However, the OVX rats after long-term absence of estrogen treated with cholecalciferol at dose of 1.0 mg/kg and low dose of 17β-E2 demonstrated lower grooming than OVX rats given with low dose of 17β-E2 alone. On the other hand, the OVX rats with 12 weeks post-OVX administered with cholecalciferol at doses 2.5 and 5.0 mg/kg/day in combination with 17β-E2 showed similar depression-like profile like the OVX rats given with 17β-E2 administration. Interestingly, cholecalciferol at dose of 2.5 mg/kg induced similar changes of the behavioral reactions in the OFT (increased motor activity and decreased grooming behavior) like administration of cholecalciferol at dose of 1.0 mg/kg/day in a combination with low dose of 17β-E2 in the OVX rats after long-term absence of estrogen. Furthermore, cholecalciferol at a dose of 5.0 mg/kg/day in combination with 17β-E2, significantly increased grooming events and failed to influence motor activity in the OVX rats in the OFT. However, we did not any and potentiation of the antidepressant-like effects of both preparations in the FST when we used cholecalciferol at doses 2.5 and 5.0 mg/kg/day SC in combination with 17β-E2. Thus, the effects of cholecalciferol at different doses alone or in a combination with a low dose of 17β-E2 on the anxiety-like or depression-like behavior of the OVX rats after long-term absence of estrogen were specific, since effects cholecalciferol on behavioral reactions did not associated with its effects on behavioral activity of these OVX rats in the OFT.

It is well-known that VD plays an important role in motor functions. VDR are widespread in the brain and the spinal cord, including the areas involved in regulation of motor activity and grooming behavior (Prufer et al., 1999; Langub et al., 2001; Walbert et al., 2001). Some data show that VDR genetic ablation produces severe motor impairment in mutant mice compared to wild-type and heterozygous control animals (Burne et al., 2005; Kalueff et al., 2004a). These impairments are likely associated with disturbed calcium homeostasis (Kalueff et al., 2004b). The

 doi:10.20388/omp2017.003.0051

results presented here also show that cholecalciferol at all tested doses increased grooming behavior in the OVX rats. However, we did not observe increase of grooming behavior in the OVX rats treated with a combination of cholecalciferol at dose of 1.0 and 2.5 mg/kg plus 17β-E2. VD has been reported to be involved in VDR-mediated modulation of brain neurotransmitters, including acetylcholine and dopamine (Carswell, 1997; Garcion et al., 2002), known to regulate grooming (Kalueff, 2002). Some study showed that VDR knockout (VDRko) mice tend to spend more time grooming than do the wild-type (WT) animals (Kalueff et al, 2004c). Such genetic ablation of VDR may affect the brain neurophysiological mechanisms and pathways that control normal grooming behavior (VanErp et al., 1994). It is there for possible to suggest that impaired VDR system in the OVX rats may result in the increased grooming seen in the present study. Further studies are needed to find how cholecalciferol might alter VDR expression and/or its sensitivity in the brain areas of the OVX involved in regulation of motor activity and grooming behavior. On the other hand, cholecalciferol at a dose of 5.0 mg/kg profoundly increased anxiety-like behavior in the OVX rats with long-term absence of estrogen. However, the OVX rats that received cholecalciferol at a dose of 5.0 mg/kg in combination with 17β-E2 showed an anxiety-like state similar to the OVX rats treated only with 17β-E2. In the present study, only one concentration of 17β-E2 was used because this concentration of 17β-E2 has been shown to exert anxiolytic-like effects in OVX rats (Fedotova et al., 2004; Fedotova, 2013). Different dosages and duration of 17β-E2 treatment should be tested in future studies.

There are some explanations for the antidepressant- and anxiolytic-like effects of cholecalciferol in the intact and OVX female rats after long-term ovariectomy. Firstly, vitamin D mediates its function via binding to VRD and the enzyme 1a-hydroxylase, which are widely located in neuronal and glial cells of the human brain (Eyles et al.,, 2013, 2014). Previous studies have found that VDR knock-out mice showed increased anxiety symptoms (Kalueff et al., 2004b). Therefore, it was speculated that defects in the vitamin D-VDR system of the OVX rats may directly result in affective-related disorders. Secondly, 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 depression and anxiety, and the beneficial effects of VD in these brain-related disorders is, at least partially, via its modulating effect on neurotransmission (Kesby et al., 2011; Jiang et al., 2013). Animal studies have suggested that VD may increase the synthesis and/or metabolism of neurotransmitters, including serotonin, dopamine and norepinephrine (Cass et al., 2014; Patrick, Ames, 2004). It was found that VD 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 up regulating glial derived neurotrophic factor (GDNF) (De Luca, 2004; Correale et al., 2009). VD has also been found to affect the expression of genes associated with γ-aminobutyric acid neurotransmission (Jiang et al., 2013) and to stimulate the expression of tyrosine hydroxylase (TH), responsible for catecholamine biosynthesis (Puchacz et al., 1996). VD responsive elements are present in the promoter regions of serotonin receptors and tryptophan hydroxylase receptors, both of which are known to be associated with emotional disorders (Fernandes de Abreu et al., 2009). Altered GABA status has been found in the brain tissues of rodents with a VD-deficient diet (Ishikawa et al., 1982; Byrne et al., 2013), and a significant reduction of glutamate decarboxylase (GAD)67 and GAD65 protein levels has been observed in adult VD deficient mice (Groves et al., 2013). Another potential explanation for antidepressant- and anxiolytic-like effects of cholecalciferol in the OVX females, especially in the OVX rats treated with low dose of 17β-E2 (the profound antidepressant- and anxiolytic-like effects) could be the following. The physiological function of VD related to the female reproductive system has recently been reported (Belkacemi et al., 2003; Avila et al., 2004; Zarinani et al., 2010). VDR is expressed in the ovaries, uterus, and decidua of the placenta. In the placenta, VDR regulates calcium transfer between trophoblasts and the endometrial decidua, which helps maintain pregnancy by preventing contraction of the uterine muscle. However, the other physiological roles of VDR in reproductive organs are not clear (Avila et al., 2004; Zarinani et al., 2010). Data in the literature suggest that there is a functional synergy between VD and 17β-E2. It was found that VD enhanced E2 biosynthesis (Nashold et al., 2009). VDR-targeted female mice had uterine hypoplasia and impaired folliculogenesis, because a lack of estrogen synthase in the ovary decreased E2 biosynthesis (Kinuta et al., 2000). Estrogen administration reversed these defects. There is also potential effect of VD on the expression of estrogen receptor alpha gene expression. Some studies in human cells have shown that VD down regulates the expression of estrogen receptor alpha gene with major impact on gene transcription (Offner, 2004; Pedersen et al., 2007). Although these findings were predominantly found in breast cancer models, it could occur in other tissues also, for example in the brain. On the other hand, E2 suppressed 1,25-dihydroxyvitamin D3 24-hydroxylase (Cyp24a1) gene expression, leading to VD accumulation, and enhancement of VDR gene expression in females (Liel et al., 1992; Duque et al., 2002). Therefore, estrogens could enhance VD synthesis by estrogen receptor-mediated down regulation of Cyp24a1 and up regulation of VDR, while VD increases estrogen biosynthesis by VDR-mediated upregulation of estrogen synthase. Moreover, some studies suggest that VD is implicated in biosynthesis of progesterone in experimental animals, and VD was shown to increase progesterone in human ovarian cells (Merhi et al., 2014). Thus, we can speculate that VD and 17β-E2 might regulate the metabolism of each other and/or estrogen receptors (ER), progesterone receptors (PR) or VDR expression in the CNS.

However, there were several limitations to the present study. The blood levels of VD and calcium were not studied in the intact and OVX rats given with different doses of VD. Detailed studies are required to explore how the blood levels of VD and calcium change after cholecalciferol treatment alone or in a combination with low dose of 17β-E2 in the intact and OVX rats. Thirdly, studies with large samples and long-term follow-up are needed to explore the expression levels for VDR, ER or PR in intact, OVX and OVX female rats treated with cholecalciferol alone or in a combination with low dose of 17β-E2. Clearly, more work is needed to understand the role of the vitamin D/VDR system in the regulation of affective-related behavior in animals and humans with imbalance of gonadal hormones. Based our results it may be suggested that cholecalciferol helps to provide significant protection against long-term ovariectomy-induced affective-related behavior. It is noteworthy to mention that, to our knowledge, this study is the first to demonstrate the ability of cholecalciferol to reduce affective-related behavior in the OVX rats after long-term ovariectomy. Further research is needed to elucidate the detailed mechanism by which cholecalciferol and 17β-E2 exert synergistic effects on anxiety-related behavior.

Conclusions

The present data of our preclinical study indicate that chronic treatment with cholecalciferol at doses of 1.0 and 2.5 mg/kg induced anxiolytic-like effects in female rats following long-term ovariectomy. The findings also suggest that the combination of cholecalciferol at doses of 1.0 and 2.5 mg/kg with a low dose of 17β-E2 more effectively decreases anxiety-like behavior in the OVX rats after long-term estrogen deficiency than17β-E2 alone. Furthermore, cholecalciferol at a dose of 1.0 mg/kg treatment decreased depression-related behavior after long-term ovariectomy, as well as the combination of cholecalciferol at a dose of 1.0 mg/kg and 17β-E2 induces a more synergic antidepressant-like effects in the OVX rats after long-term ovariectomy. Furthermore, this is the first study to show a beneficial effect of chronic treatment with cholecalciferol at doses of 1.0 and 2.5 mg/kg on affective-related state induced by long-term ovariectomy in female rats.

Acknowledgments

The reported study was funded by Russian Science Foundation (RSF) accordingly to the research project No. 16-15-10053.

Conflict of Interest

The authors declare no conflict of interest.

References

AVILA E., DÍAZ L., HALHALI A., LARREA F. (2004). Regulation of 25-hydroxyvitamin D3 1α-hydroxylase, 1,25-dihydroxyvitamin D3 24-hydroxylase and vitamin D receptor gene expression by 8-bromo cyclic AMP in cultured human syncytiotrophoblast cells. Steroid Biochem Mol Biol, 89, 115-119.

BELKACEMI L., GARIÉPY G., MOUNIER C., SIMONEAU L., LAFOND J. (2003). Expression of calbindin-D28k (CaBP28k) in trophoblasts from human term placenta. Biol Reprod, 68, 1943-1950.

BOSEE R., DI PAOLO T. (1995). Dopamine and GABAA receptor imbalance after ovariectomy in rats: Model of menopause. J Psychiatr Neurosci, 20, 364-371.

BROMBERG J., KRAVITZ H., CHANG Y., RANDOLPH J.J., AVIS N., GOLD E., MATTEWS K. (2013). Does risk for anxiety increase during the menopausal transition? Study of women's health across the nation. Menopause, 20, 488-495.

BURGER H. (2008). The menopausal transition - endocrinology. J Sex Med, 5, 2266-2273.

BURNE T.H., MCGRATH J.J., EYLES D.W., MACKAY-SIM A. (2005). Behavioural characterization of vitamin D receptor knockout mice. Behav Brain Res, 157, 299-308.

BYRNE J.H., VOOGT M., TURNER K.M., EYLES D.W., MCGRATH J.J., BURNE T.H. (2013). The impact of adult vitamin D deficiency on behaviour and brain function in male Sprague-Dawley rats. PLoS ONE, 8.

CASS W.A., PETERS L.E., FLETCHER A.M., YUREK D.M. (2014). Calcitriol promotes augmented dopamine release in the lesioned striatum of 6-hydroxydopamine treated rats. Neurochem Res, 39, 1467-1476.

CARSWELL S. (1997). Vitamin D in the Nervous System: Actions and Therapeutic Potential. In Vitamin D; Feldman, D., Glorieux, F.H., Pike, J.W., Eds.; Academic Press: San Diego, CA, USA, 1997; pp. 1197-1211.

CORREALE J., YSRRAELIT M.C., GAITÁN M.I. (2009). Immunomodulatory effects of vitamin D in multiple sclerosis. Brain, 132, 1146-1160.

DE LUCA H.F. (2004). Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr, 80, 1689-1696.

DUQUE G.K., El ABDAIMI M., MACORITTO M., MILLER M., KREMER R. (2002). Estrogens (E2) regulate expression and response of 1,25-dihydroxyvitamin D3 receptors in bone cells: Changes with aging and hormone deprivation. Biochem Biophys Res Commun, 299, 446-454.

ESTRADA-CAMARENA E., FERNANDEZ-GUASTI A., LOPEZ-RUBALCAVA C. (2004). Interaction between estrogens and antidepressants in the forced swimming test in rats. Psychopharmacol, 173, 139-145.

ESTRADA-CAMARENA E., LOPEZ-RUBALCAVA C., AZUCENA HERNANDEZ-ARAGON A., SILVIA MEJIA-MAURIES S., PICAZO O. (2017). Long-term ovariectomy modulates the antidepressant-like action of estrogens, but not of antidepressants. J Psychopharmacol, 25, 1365-1377.

EYLES D.W., SMITH S., KINOBE R., HEWISON M.,

 doi:10.20388/omp2017.003.0051

MCGRATH J.J. (2005). Distribution of the vitamin D receptor and 1a-hydroxylase in human brain. J Chem Neuroanat, 29, 21-30.

EYLES D.W., BURNE T.H., MCGRATH J.J. (2013). Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front. Neuroendocrinol, 34, 47-64.

EYLES D.W., LIU P.Y., JOSH P., CUI X. (2014). Intracellular distribution of the vitamin d receptor in the brain: Comparison with classic target tissues and redistribution with development. Neurosci, 268, 1-9.

FEDOTOVA J., HARTMANN G., LENARD G., SAPRONOV N. (2004). Effects of 5-HT1A receptor agonist and antagonist on anxiety in intact and ovariectomized female rats. Acta Physiol Hung, 91, 175-184.

FEDOTOVA J. (2013). Anxiolytic-like effect of quinpirole in combination with a low dose of 17β-estradiol in ovariectomized rats. Acta Physiol Hung, 100, 211-223.

FEDOTOVA J., SOULTANOV V., NIKITINA T., ROSCHIN V. & ORDAYN N. (2012). Ropren® is a polyprenol preparation from coniferous plants that ameliorates cognitive deficiency in a rat model of beta-amyloid peptide-(25–35)-induced amnesia. Phytomed, 19, 451-456.

FERNANDES DE ABREU D.A., EYLES D., FÉRON F. (2009). Vitamin D, a neuroimmunomodulator: Implications for neurodegenerative and autoimmune diseases. Psychoneuroendocrinol, 34, S265-S277.

GARCION E., WION-BARBOT N., MONTERO-MENEI C., BERGET F., WION D. (2002). New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab, 13, 100-105.

GROVES N.J., KESBY J.P., EYLES D.W., MCGRATH J.J. MACKAY-SIM A., BURNE T.H. (2013). Adult vitamin D deficiency leads to behavioural and brain neurochemical alterations in C57BL/6J and BALB/c mice. Behav Brain Res, 241, 120-131.

JIANG P., ZHANG W.Y., LI H.D., CAI H.L., XUE Y. (2013). Repeated haloperidol administration has no effect on vitamin D signaling but increase retinoid X receptors and Nur77 expression in rat prefrontal cortex. Cell Mol Neurobiol, 33, 309-312.

IDRUS N.M., HAPPER J.P., THOMAS J.D. (2013). Cholecalciferol attenuates perseverative behavior associated with developmental alcohol exposure in rats in a dose-dependent manner. J Steroid Biochem Mol Biol 136, 146-149.

ISHIKAWA K., OTT T., MCGAUGH J.L. (1982). Evidence for dopamine as a transmitter in dorsal hippocampus. Brain Res, 232, 222-226.

KALUEFF A.V. (2002). Grooming and Stress; Kiv, Ed.; KSF Publishers: Kimberley, Australia.

KALUEFF A.V., LOU Y.R., LAAKSI I., TUOHIMAA P. (2004a). Impaired motor performance in mice lacking neurosteroid vitamin D receptors. Brain Res Bull, 64, 25-29.

KALUEFF A.V., LOU Y.R., LAAKSI I., TUOHIMAA P. (2004b). Increased anxiety in mice lacking vitamin D receptor gene. Neuroreport, 15, 1271-1274.

KALUEFF A.V., LOU A.Y.-R., LAAKSIB I., TUOHIMAA A P. (2004c). Increased grooming behavior in mice lacking vitamin D receptors. Physiol Behav, 82, 405-409.

KESBY J.P., EYLES D.W., BURNE T.H., MCGRATH J.J. (2011). The effects of vitamin D on brain development and adult brain function. Mol Cell Endocrinol 347, 121-127.

Kinuta, K.; Tanaka, H.; Moriwake, T.; Aya, K.; Sato, S.; Seino, Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology 2000, 141, 1317-1324.

LANGUB M.C., HERMAN J.P., MALLUCHE H.H., KOSZEWSKI N.J. (2001). Evidence of functional vitamin D receptors in rat hippocampus. Neurosci, 104, 49-56.

LIEL Y., KRAUS S., LEVY J., SHANY S. (1992). Evidence that estrogens modulate activity and increase the number of 1,25-dihydroxyvitamin D receptors in osteoblast-like cells (ROS 17/2.8). Endocrinol, 130, 2597-2601.

MARSHALL K.M. (2011). Introduction to the interaction between gonadal steroids and the central nervous system. Curr Top Behav Neurosci, 8, 1-13.

MENZAGHI F., HOWARD R.L., HEINRICHS S.C., VALE W., RIVIER J., KOOB G.F. (1994). Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J. Pharmacol Exp Ther, 269, 564-572.

MERHI Z., DOSWELL A., KREBS K., CIPOLLA M. (2014). Vitamin D alters genes involved in follicular development and steroidogenesis in human cumulus granulosa cells. J Clin Endocrinol Metab, 99, E1137-E1145.

NASHOLD F.E., SPACH K.M., SPANIER J.A., HAYES C.E. (2009). Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression. J Immunol, 183, 3672-3681.

NELLY M., RIVERA V., TENORIO A.G., FERNÁNDEZ-GUASTI A., ESTRADA-CAMARENA E. (2016). The post-ovariectomy interval affects the antidepressant-like action of citalopram combined with ethynyl-estradiol in the forced swim test in middle aged rats. Pharmaceuticals, 9, 21.

OFFNER H. (2004). Neuroimmuno protective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: Therapeutic implications for multiple sclerosis. J Neurosci Res 2004, 78, 603-624.

OKADA M., HAYASHI N., KOMETANI M., NAKAO K., INUKAI T. (1997). Influences of ovariectomy and continuous replacement of 17β-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. Jpn J Pharmacol 1997, 73, 93-96.

PATRICK R.P., AMES B.N. (2014). Vitamin D hormone regulates serotonin synthesis. Part 1: Relevance for

autism. FASEB J, 28, 2398-2413.
PEDERSEN L.B., NASHOLD F.E., SPACH K.M., HAYES C.E. (2007). 1,25-Dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J Neurosci Res, 85, 2480-2490.
PELLOW S., FILE S.E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol Biochem Behav, 24, 526-530.
PENG W., SIBBRITT D.W., HICKMAN L., ADAMS J. (2016). Association between use of self-prescribed complementary and alternative medicine and menopause-related symptoms: a cross-sectional study. Complement Ther Med, 23, 666-673.
PICAZO O., ESTRADA-CAMARENA E., HERNANDEZ-ARAGON A. (2006). Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioral actions of some anxiolytic agents. Eur J Pharmacol, 530, 88-94.
PICK U., HARAMAKI N., CONSTANTINESCU A., HANDELMAN G.J., TRITSCHLER H.J., PACKER L. (1995). Glutathione reductase and lipoamide dehydrogenase have opposite stereospecificities for α-lipoic acid enantiomers. Biochem Biophys Res Commun 206, 724-730.
PORSOLT R.D., ANTON G., BLAVET N., JALFRE M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol, 47, 379-391.
PRUFERK., VEENSTRAT.D., JIRIKOWSKIG.F., KUMAR R. (1999). Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord. J Chem Immunol, 16, 135-145.
PUCHACZ E., STUMPF W.E., STACHOWIAK E.K., STACHOWIAK M.K. (1996). Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. Brain Res Mol Brain Res, 36, 193-196.
SCHEID V., WARD T., CHA W.S., WATANABE K., LIAO X. (2010). The treatment of menopausal symptoms by traditional East Asian medicines: Review and perspectives. Maturitas. 66, 111-130.
SOARES C.N., MAKI P.M. (2010). Menopausal transition, mood, and cognition: An integrated view to close the gaps. Menopause, 17, 812-814.
SOARES C.N. (2013). Depression in peri- and postmenopausal women: Prevalence, pathophysiology and pharmacological management. Drugs Aging, 30, 677-685.
STANZIONE P., CALABRESI P., MERCURI N., BEMARDI G. (1984). Dopamine modulates CA1 hippocampal neurones by elevating the threshold for spike generation: an in vitro study. Neurosci, 13, 1105-1116.
STEWART A., WONG K., CACHAT J., ELEGANTE M., GILDER T., MOHNOT S., WU N., MINASYAN, A.; TUOHIMAA P., KALUEFF A.V. (2010). Neurosteroid vitamin D system as a nontraditional drug target in neuropsychopharmacology. Behav Pharmacol 21, 420-426.
VANERP A.M.M., KRUK M.R., MEELIS W., WILLEKENS-BRAMER D.C. (1994). Effect of environmental stressors on time course, variability and form of self-grooming in the rat: Handling, social contact, defeat, novelty, restraint and fur moistening. Behav Brain Res, 65, 47-55.
VERA P.G., RADA G.C. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. Obstet Gynecol Syrv, 57, 750-752.
WALBERT T., JIRIKOWSKI G.F., PRUFER K. (2001). Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the limbic system. Horm Metab Res, 33, 525-531.
WANG J.Y., WU J.N., CHERNG T.L., HOFFER B.J., CHEN H.H., BORLONGAN C.V., WANG Y. (2001). Vitamin D3 attenuates 6-hydroxydopamine-induced neurotoxicity in rats. Brain Res 904, 67-75.
WRZOSEK M., LUKASZKIEWICZ J., JAKUBCZYK A., MATSUMOTO H., PIATKIEWICZ P., RADZIWONZALESKA M., WOJNAR M., NOWICKA G. (2013). Vitamin D and the central nervous system. Pharmacol Rep, 65, 271-278.
ZARINANI A.H., SHAHBAZI M., SALEK-MOGHADDAM A., ZAREIE M., TAVAKOLI M., GHASEMI J., REZANIA S., MORAVEJ A., TORKABADI E., RABBANI H. (2010). Vitamin D3 receptor is expressed in the endometrium of cycling mice throughout the estrous cycle. Fertil Steril, 93, 2738-2743.