Post-Ovariectomy Period Influences Depression-Like Behavior in the Adult Female Rats Treated with Different Doses of Cholecalciferol

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Abstract. The present study was performed to determine the behavioral effects of cholecalciferol (Vitamin D3) hormone treatment at different doses as an adjunctive therapy alone or in a combination with low dose of 17β-estradiol on depression-like behavior of female rats after long-term absence of estrogen. The aim of the study was to examine the effects of chronic cholecalciferol administration (1.0, 2.5 or 5.0 mg/kg/day, SC once daily, for 14 days) on depression-like behavior following long-term ovariectomy (12 weeks) of the adult (3 months old) female rats of Wistar line. Cholecalciferol was administered to the ovariectomized (OVX) rats and OVX rats treated with low dose of 17β-estradiol (17β-E2, 0.5 μg/rat, SC once daily, for 14 days) after long-term ovariectomy. Depression-like behavior was assessed in the forced swimming test (FST), locomotor and grooming activities were assessed in the open field test (OFT). Using biochemical studies were evaluated estradiol and 25-hydroxyvitamin D3 levels in the blood serum of OVX rats treated with cholecalciferol alone and cholecalciferol plus 17β-E2. Chronic administration of cholecalciferol (5.0 mg/kg/day, SC) into the intact females significantly reduced depression-like behavior in the FST ($p<0.05$). The treatment with cholecalciferol (1.0 mg/kg/day, SC) in the OVX rats after long-term absence of estrogens induced antidepressant-like effect ($p<0.05$) in the FST. Moreover, cholecalciferol in this dose plus 17β-E2 more markedly exhibited antidepressant-like effect in the OVX rats after long-term ovariectomy ($p<0.05$). Simultaneously, treatment with cholecalciferol (1.0 mg/kg/day, SC) in the OVX rats after long-term absence of estrogens produced elevated estradiol and 25-OH-VD3 levels for the OVX rats as compared to the OVX females. The combined application of cholecalciferol (2.5 and 5.0 mg/kg/day, SC) and 17β-E2 produced antidepressant-like effect that was similar to the antidepressant-like effect of 17β-E2. Our results indicate that cholecalciferol at dose of 5.0 mg/kg/day induced antidepressant-like effect only in intact rats subjected FST. Following long-term ovariectomy in the adult female rats, cholecalciferol at dose of 1.0 mg/kg/day administered alone resulted in decrease of depression-like behavior in the FST. Moreover, cholecalciferol at dose of 1.0 mg/kg/day in a combination with 17β-E2 at a low dose induced synergic antidepressant-like effect in the FST.

Keywords: cholecalciferol; vitamin D3; depression; behavior; estradiol; long-term ovariectomy.

Introduction

Women's transition into reproductive senescence is marked by reductions in ovarian function and output, referred to as menopause (Santoro et al., 2015). This stage is characterized by a dramatic development of affective-related disorders and different psychoemotional pathologies (Maki et al., 2010). A strategy to alleviate the mood disorders associated with menopause is hormonal replacement therapy (HRT) (Soares et al., 2013). However, controversial results related to the effectiveness of such treatment have been frequently reported (Soares et al., 2001). These discrepancies could be associated to various factors, one of them being the time when estrogen restitution is initiated after the beginning of menopause (Pae et al., 2009; Soares et al., 2010; Vedder et al., 2014).

On the other hand, antidepressant application is an important approach to treat depression in women, however, not all individuals respond to pharmacologic therapy and the management of depression in these patients is complicated by comorbid medical conditions, potential drug interactions, increased vulnerability to side effects of medications usually used to treat depression, and drug costs (Amsterdam et al., 1999; Soares, Maki, 2010; Studd, Nappi, 2012). Therefore, the search of novel interventions oriented toward treatment for depression among women with menopausal period may provide a reasonable alternative (Soares, Maki, 2012).

There has been longstanding interest in the role of «natural» treatments for depression, such as nutritional and dietary products. While many dietary factors have been implicated in the cause and treatment of depression, there has been a lack of scientific rigour in many of the reported studies (Scheid et al., 2010; Peng et al., 2016). Among other nutraceuticals, one of such «natural» substances for treatment of depression could be vitamin D (VD) (Genaro et al., 2007; Studd, Nappi, 2012). VD which comprises a group of fat-soluble secosterols found in very few foods, is photosynthesized in the skin of vertebrates by the action of solar ultraviolet B radiation (UV-B) (Plum, DeLuca, 2010; Christakos, 2010). The major biological function of VD is the maintenance of normal concentrations of serum calcium and phosphorus by enhancing the efficiency of the small intestine to absorb these minerals from food (Christakos,

DeLuca, 2011).

Through decades VD was considered a vitamin, but nowadays it has emerged as an active hormone exerting its action as a transcription factor regulating the expression of numerous genes (Theodoratou et al., 2014). Vitamin D enters the blood stream and is metabolised in the liver, forming 25-hydroxyvitamin D or 25 (OH) D and is then further metabolised in the kidneys to its active form (1,25-dihydroxyvitamin D) (Deluca, 2014; Heaney, 2008). It then binds to vitamin D receptors (VDR) in target tissues to regulate gene transcription and to structures within cell membranes to mediate a number of non-genomic responses (Eyles et l., 2003; Blomberg et al., 2010).

VDR are present in most tissues and cells in the body, and within the brain show some specificity to the prefrontal cortex, hippocampus, cingulate gyrus, thalamus, hypothalamus and substantia nigra (Eyles et al., 2005; Eyles et al., 2013, 2014). This is of relevance as many of those brain regions have been implicated in the physiology of depression (Drevets et al., 2008). However, the role of VDR in the pathology of depression has not been clearly established.

There was found that estrogen deficiency effects on depressive-like behavior are restricted to certain periods after ovary removal (de Chaves et al., 2009; Estrada-Camaerena E. et al., 2017). Moreover, preclinical data suggest that time frame after ovariectomy can be important to obtain behavioral positive or negative results with HRT alone or in a combination with some existing antidepressants (Nelly et al., 2016).

We have previously shown that chronic administration of cholecalciferol at dose of 5.0 mg/kg, SC had a marked antidepressant-like effect in the adult female rats after 2 weeks of post-ovariectomy (Fedotova et al., 2016). We assumed the following: 1) antidepressant-like effects of cholecalciferol in OVX rats after 12 weeks post-ovariectomy can be differed from its effects in OVX rats after 2 weeks post-ovariectomy; 2) different doses of cholecalciferol in OVX rats with 12 weeks post-ovariectomy interval might lead to negative versus positive effects which are similar or contrast to the effects for different doses of cholecalciferol in OVX rats with 2 weeks post-ovariectomy. To our knowledge, there are no studies analyzing the behavioral effects of cholecalciferol in the OVX rats after short- and long-term period. Thus, it is a great interest to evaluate the effects of repeated cholecalciferol administration on depression-related behavior in the adult female rats with long-term estrogen deficiency.

The aim of the present study was: (1) to determine if repeated systemic treatment with cholecalciferol affected on depression-related behavior in female rats after long-term ovariectomy, (2) to clarify whether after repeated treatment of cholecalcifeol, its effects on depression-like behavior may be determined and depended from the hormonal state of female rats (low estrogen level or 17β-estradiol application), (3) to investigate whether effects of repeated treatment of cholecalcifeol alone or plus 17β-estradiol (17β-E2) on the depression-like behavior of female rats after 12 weeks post-ovariectomy period could differ from its effects in the female rats after 2 weeks post-ovariectomy period.

Materials and Methods

Animals

The study used 96 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 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

 doi:10.20388/omp2018.001.0056

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 (20). 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 (sham-operated) females treated with sterile water with 2% ethanol as solvent for cholecalciferol in behavioral tests for measurement of depression-like state (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 SC (intact rats + cholecalciferol 1.0 mg/kg), cholecalciferol at a daily dose of 2.5 mg/kg SC (intact rats + cholecalciferol 2.5 mg/kg) or cholecalciferol at a daily dose of 5.0 mg/kg SC (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). All experimental groups are presented in the Figure 1.

To summarize the treatment workflow, after induction of the experimental model of estrogen deficiency, the OVX

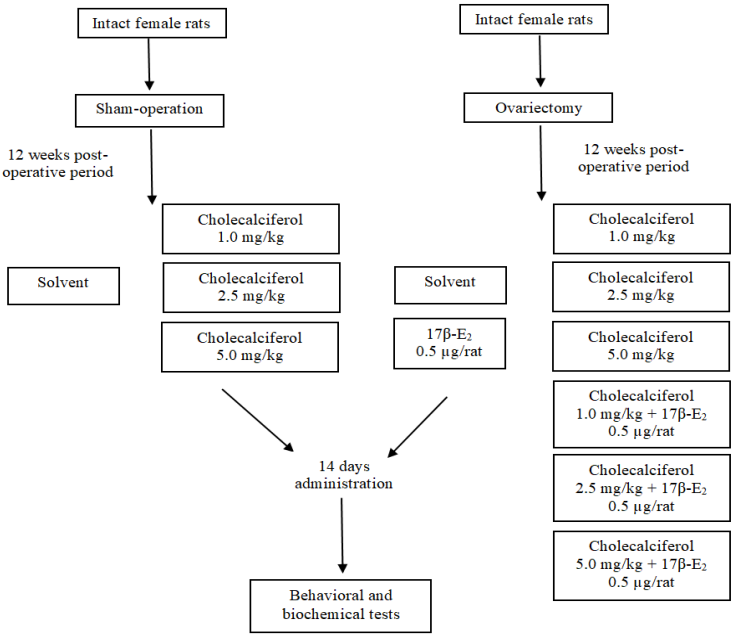

Figure 1. *The general scheme of experiment. All experimental groups used in the behavioral and biochemical experiments are presented.*

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. 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. 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.

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.

Biochemical studies

Estradiol status

Approximately 5 ml of blood samples were drawn from animals anesthetized with ketamine (5.0-10 mg/kg, i.m.). After centrifugation, plasma samples were used for the measurement of estradiol levels using a commercially available ELISA kit (DRG Diagnostics, Marburg, Germany). The sensitivity of the ELISA was 3.0 pg/ml.

Vitamin D3 status

Approximately 5 ml of blood samples were drawn from animals anesthetized with ketamine (5.0-10 mg/kg, i.m.). After centrifugation, serum samples were frozen at -200C until analysis. Afterthat, serum samples were used for the measurement of Rat 25-hydroxyvitamin D3 (25-OH-VD3) levels using a commercially available ELISA kit (CSB-E08098r, Cusabio Biotech Co., Ltd, Wuhan, P.R. China). Technical variability was low with coefficients of variation of <10% intra-assay and <15% inter-assay. Detection range is 20 μg/L-100 μg/L. The sensitivity of the ELISA was 5.0 μg/L.

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 condition 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 both OVX females and OVX females treated with 17β-estradiol following long-term absence of estrogen 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 ($p < 0.05$).

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

 doi:10.20388/omp2018.001.0056

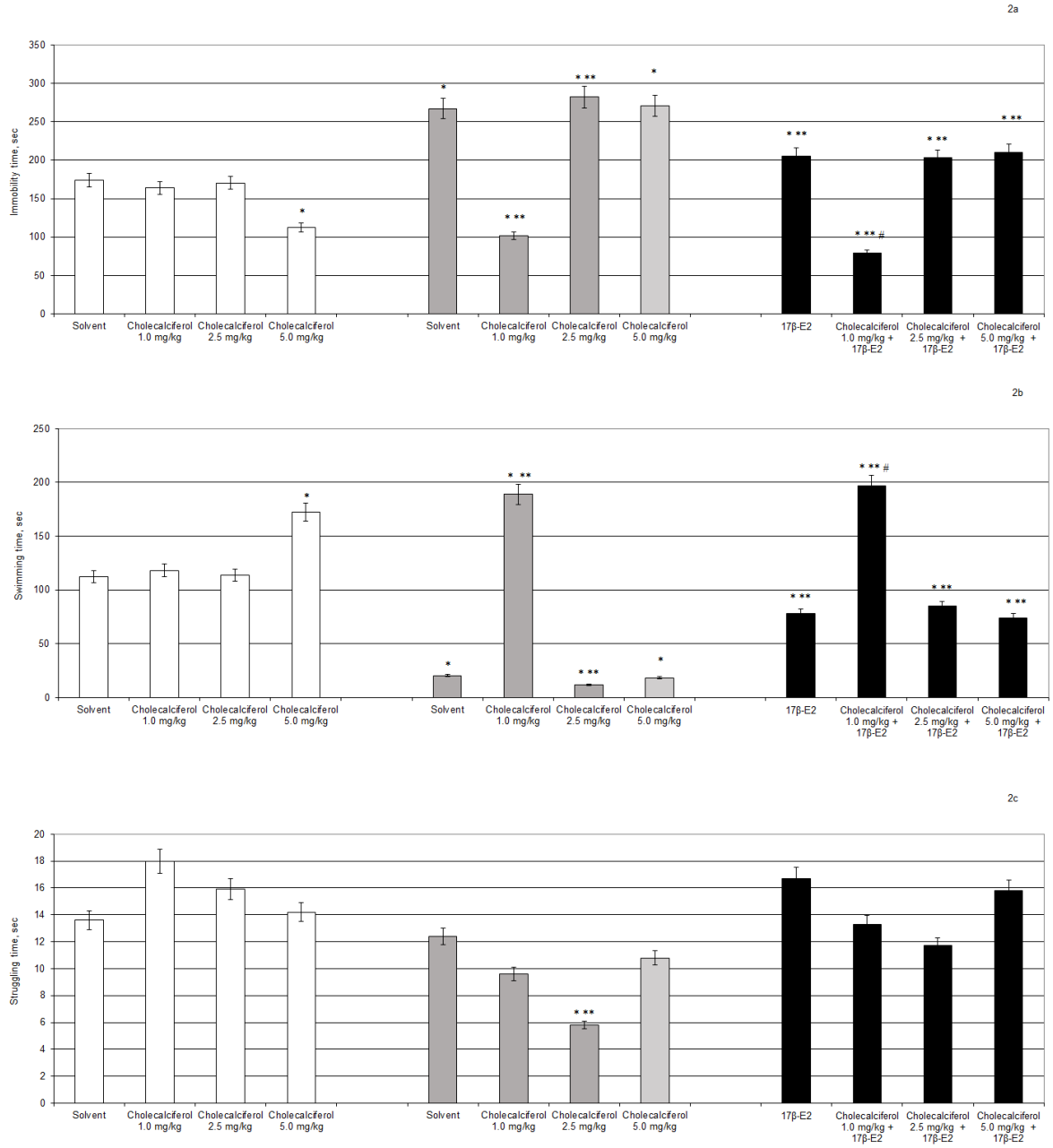

Figure 2. *Effects of cholecalciferol administration on depression-like behavior of ovariectomized (OVX) rats following long-term estrogen deficiency in the forced swimming test. (a) – immobility time, sec; (b) – swimming time, sec; (c) – struggling time, sec. The obtained results show the mean ± standard error of the mean (SEM).*– 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-E2. Each group comprised a minimum of eight rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day SC, once daily, for 14 days. The administered dose of 17β-E2 was 0.5 μg/rat SC, once daily, for 14 days.*

control rats (Figure 2a, $p > 0.05$). 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 2a, $p < 0.05$).

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 2a, $p < 0.05$). 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 2a, $p < 0.05$). 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 2a, $p < 0.05$). 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 2a, $p < 0.05$). Cholecalciferol treatment (5.0 mg/kg) failed to modify immobility time in the FST as compared to the OVX/solvent rats (Figure 2a, $p > 0.05$).

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 2a, 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 2a, 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 2b, 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 2b, 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, Figure 2b). 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 2b). 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 2b, 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 2b, 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 2b, 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 2b, 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 2b, 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 2c, 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 2c).

Effects of Cholecalciferol administration on behavioral alterations of both OVX females and OVX females treated with 17β-estradiol following long-term absence of estrogen 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 1, 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 1, p > 0.05).

OVX rats given with solvent exhibited a significant decrease of grooming behavior as compared to the control rats (Table 1, 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 1, 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 1, p < 0.05). However, cholecalciferol administration in all doses (1.0, 2.5 and 5.0 mg/kg SC) to the OVX rats resulted in a significant elevated frequency of grooming as compared to the OVX rats treated with solvent (Table 1, 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 (Table1, 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 1, p< 0.05). Cholecalciferol at a dose of 5.0 mg/kg in a combination with 17β-E2 in the OVX rats

 doi:10.20388/omp2018.001.0056

Table 1

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** #

The obtained results show the mean ± standard error of the mean (SEM).*– $p<0.05$ 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 SC, once daily, for 14 days. The administered dose of 17β-E2 was 0.5 µg/rat SC, once daily, for 14 days.

failed to induce any changes of locomotor activity when these rats were compared to the OVX rats treated with 17β-E2 (Table 1, $p > 0.05$).

Effects of Cholecalciferol administration on 25-hydroxyvitamin D3 and estradiol levels in the blood serum of both OVX females and OVX females treated with 17β-estradiol following long-term absence of estrogen

A two-way ANOVA revealed significant differences in 25-hydroxyvitamin D3 (25-OH-VD3) and estradiol levels between hormone conditions ([$F(5,32) = 11.32$, $p<0.01$ and [$F(5,32) = 23.03$, $p<0.05$], respectively), between drug treatments [$F(5,32) = 7.4$, $p<0.05$] and [$F(5,32) = 4.88$, $p<0.01$], respectively), and an interaction between hormone condition and treatments ([$F(5,32) = 16.33$, $p<0.01$] and $F(5,32) = 14.11$, $p<0.05$], respectively) in the OVX rats with long-term estrogen deficiency. The post-hoc test revealed differences among the experimental groups for 25-OH-VD3 and estradiol levels ($p<0.01$ and $p<0.05$, respectively).

The intact rats treated with cholecalciferol at doses of 1.0, 2.5 and 5.0 mg/kg dose-dependent increased 25-OH-VD3 levels and failed to alter estradiol levels in the serum blood as compared to the control rats (Figure 3ab, $p > 0.05$).

Long-term ovariectomy in female rats resulted in a significant decrease of estradiol and 25-OH-VD3 levels in the blood as compared to the control females (Figure 3ab, $p < 0.05$). The 17β-E2 supplementation (0.5 µg/kg, SC) failed to modify 25-OH-VD3 level in the blood of the OVX rats as compared to the OVX rats administered with solvent (Figure 3ab, $p > 0.05$). The value of this parameter in the OVX/17β-E2 females were lower than that of the value of control rats. However, 17β-E2 supplementation significantly increased estradiol level in the blood of the OVX rats as compared to the OVX rats given with solvent (Figure 3a, $p > 0.05$).

The OVX rats treated with cholecalciferol at doses of 1.0, 2.5 and 5.0 mg/kg dose-dependent significantly increased 25-OH-VD3 and estradiol levels in the serum blood as compared to the OVX rats treated with solvent (Figure 3ab, $p > 0.05$). However, the value of 25-OH-VD3 content in the OVX rats treated with cholecalciferol at doses of 1.0, 2.5 and 5.0 mg/kg were lower than that of the value of control rats.

Cholecalciferol treatment at doses of 1.0 and 2.5 mg/kg in combination with 17β-E2 more significantly elevated the estradiol and 25-OH-VD3 levels for the OVX rats as compared to the OVX females treated with oil solvent or 17β-E2 (Figure 3ab, $p<0.05$). Combined administration of cholecalciferol at a dose of 5.0 mg/kg and 17β-E2 in the OVX rats failed to change 25-OH-VD3 level as compared to the OVX rats administered with solvent (Figure 3b, $p > 0.05$). However, we found that cholecalciferol at a dose of 5.0 mg/kg in combination with 17β-E2 significantly increased estradiol levels when OVX rats/cholecalciferol 5.0 mg/kg + 17β-E2 rats were compared with the intact/solvent, OVX/solvent and OVX/17β-E2 rat groups (Figure 3a, $p > 0.05$).

Discussion

We examined the effects of chronic cholecalciferol treatment at different doses (1.0, 2.5 and 5.0 mg/kg SC) for 14 days on depression-like behavior in female rats with long-term estrogen deficiency and 17β-E2 supplementation in a low dose. Endogenous estrogens were removed by ovariectomy and only after 12 weeks post-ovariectomy period, these rats were used in all experiments. The results of behavioral testing for the depression-related effects of cholecalciferol were compared in both OVX rats and OVX female rats treated with 17β-E2. Simultaneously, the effects of cholecalciferol at similar doses on depression-like behavior were tested in intact female rats. For this purpose, the forced swimming test (FST) was performed in this study. We also investigated whether the effects of cholecalciferol at different doses were specific in the FST, measuring its

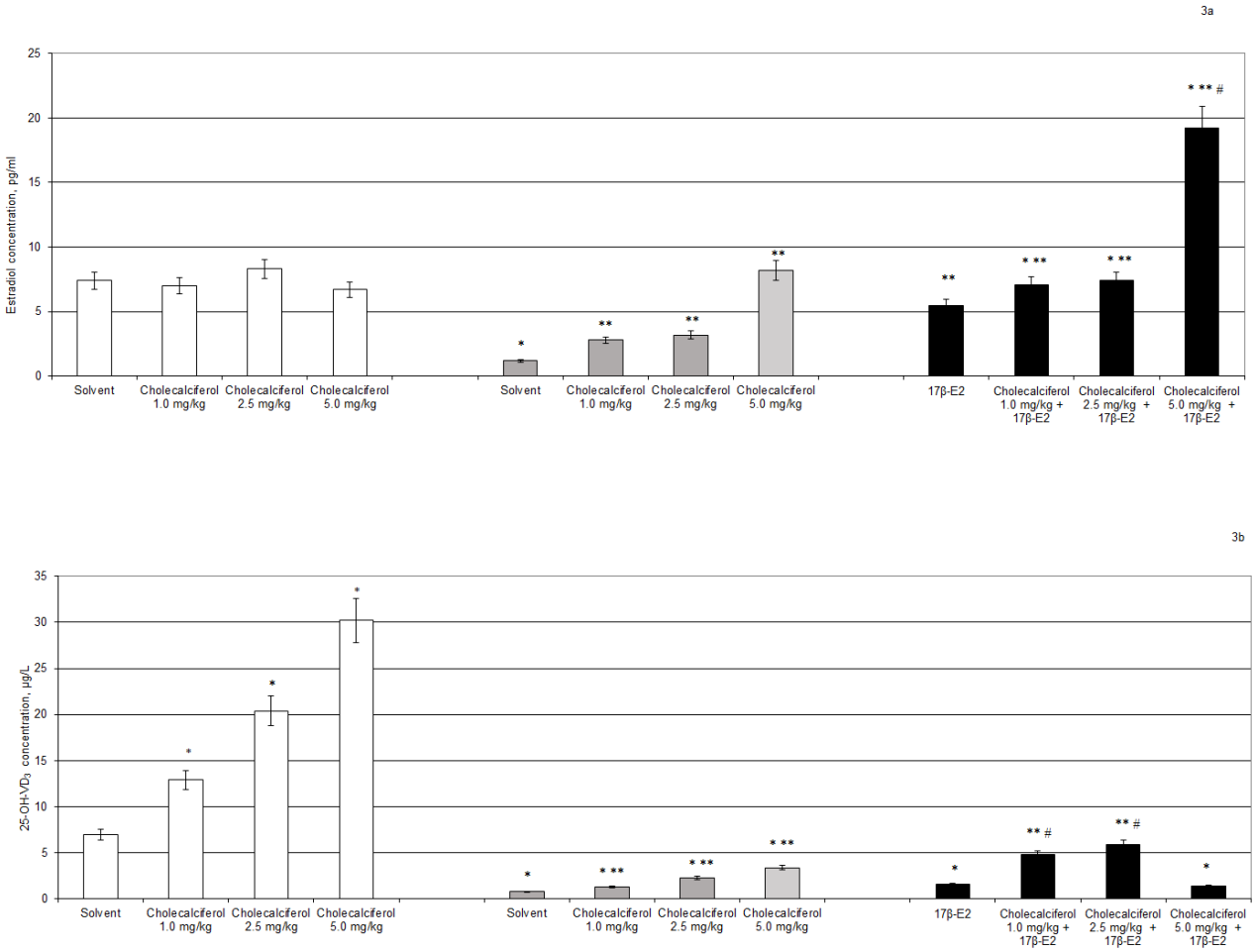

Figure 3. *Effects of cholecalciferol administration 25-hydroxyvitamin D3 and estradiol levels in the blood serum of ovariectomized (OVX) rats following long-term estrogen deficiency. (a) – estradiol concentration, pg/ml; (b) – 25-hydroxyvitamin D3 concentration, µg/L. White columns – intact female rats treated with solvent or cholecalciferol at different doses; Grey columns – OVX female rats treated with solvent or cholecalciferol at different doses; Dark columns – OVX female rats treated with 17β-E2 alone or combination of 17β-E2 and cholecalciferol at different doses. The obtained results show the mean ± standard error of the mean (SEM). *– 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-E2. Each group comprised a minimum of eight rats. Cholecalciferol was given at 1.0, 2.5 or 5.0 mg/kg/day SC, once daily, for 14 days. The administered dose of 17β-E2 was 0.5 µg/rat SC, once daily, for 14 days.*

effects on the behavioral activity in the OFT of the intact and OVX rats after long-term absence of estrogen.

Cholecalciferol at dose of 5.0 mg/kg induced decrease of depression-like behavior of intact female rats in the FST. Interestingly, in the present study cholecalciferol exhibited antidepressant-like effect only at a dose of 5.0 mg/kg SC in intact rats. It should be noted that the age of these rats was 6.5 months age at the starting of the behavioral experiments. In contrast, to our previous study where intact rats were 3.0 months of age and we demonstrated antidepressant-like effect of cholecalciferol at doses of 2.5 and 5.0 mg/kg, SC in the FST.

Analyzing the results from biochemical assay, we found dose-dependent increase of 25-OH-VD3 concentration in accordance to the corresponding dose of cholecaciferol application and absence of any modifications of estradiol level in the serum blood of intact rats given with different doses of cholecalciferol. It can be assumed that antidepressant-like effect of cholecalciferol at a dose of 5.0 mg/kg SC is associated with the VD-induced changes of hormonal state (high level of 25-OH-VD in the blood serum) of intact-ovary rats. Antidepressant-like effect of cholecalciferol at a dose of 5.0 mg/kg SC in the intact rats was not induced by changes in grooming and motor function, because the general locomotor activity and grooming reactions of these rats activity were not altered in the OFT. On the other hand, although cholecalciferol at a dose of 1.0 mg/kg resulted in a significant decrease of grooming in the females, however, we did not examine any manifestation of changes in the depression-like behavior of such group of rats. 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

 doi:10.20388/omp2018.001.0056

different doses in the FST is also needed to be done.

Our results showed that in OVX rats following 12 weeks of postovariectomy (post-OVX) period, there were marked depression-like behavior as assessed by FST. Although 17β-E2 supplementation resulted in significant antidepressant-like effect of OVX rats with long-term absence of estrogen, the 17β-E2 administration was not able to completely diminish depression-like behavior 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 depression-like behavior to some extent. In fact, this experiment showed that the effects of 17β-E2 supplementation on 25-OH-VD3 content did not associated with its effects on depression-like behavior in OVX rats. The long-term effect of ovariectomy on depression-like behavior in rats that were submitted in a standard behavioral tests (Okada et al., 1997; Nelly et al., 2016; Estrada-Camarena et al., 2017). They found that rats with 12-weeks post-OVX showed greater parameters of depression-like state than rats with 3 weeks post-OVX.

We found that cholecalciferol at dose of 1.0 mg/kg/day SC 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. 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 depression-like behavior of these rats were completely different in the FST. These data suggested that the completely different effects of cholecalciferol application at doses of 1.0, 2.5 and 5.0 mg/kg SC (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 antidepressant-like activity 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 FST test. ELISA assay demonstrated that administration of cholecalciferol in different doses resulted in a dose-dependent increase of 25-OH-VD3 levels in the blood serum of the OVX rats with long-term absence of estrogen. Cholecalciferol administered at 1.0 and 2.5 mg/kg SC similarly increased estradiol levels in the blood serum of OVX rats after long-term ovariectomy. Interestingly, that cholecalciferol at a dose of 5.0 mg/kg SC induced more profound increase of estradiol level in the OVX rats with long-term absence of estrogen, however, we did not find any modifications of the depression-like behavior in the FST of these rats. It can be supposed that there exist an «optimal» of VD and estradiol levels in the blood to produce antidepressant-like effect of cholecalciferol in the OVX rats with long-term absence of estrogen.

Interestingly, in the present study cholecalciferol exhibited antidepressant-like effect only at a dose of 1.0 mg/kg SC in the OVX rats with long-term absence of estrogen. In contrast, to our previous study where OVX rats after 2 weeks of post-OVX, the dose of 1.0 mg/kg SC of cholecalciferol was not effective and we demonstrated antidepressant-like effect of cholecalciferol only at dose of 5.0 mg/kg, SC in the FST.

Administration of cholecalciferol at dose of 1.0 mg/kg/day SC 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. 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 SC 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 SC 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 SC 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.

In fact, the effects of cholecalciferol at different doses alone or in a combination with a low dose of 17β-E2 on the 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 (Langub et el., 2001; Prufer et al., 1999; Walbert et al., 2001). Some data show that VDR genetic ablation produces severe behavioral alterations (Burne et al., 2005; Kalueff et al., 2004a). These impairments are likely

associated with disturbed calcium homeostasis Kalueff et al., 2004a). VD has been reported to be involved in VDR-mediated modulation of brain neurotransmitters, including acetylcholine and dopamine Carswell, 1997; Garcion et al., 2002; 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., 2004a). Such genetic ablation of VDR may affect the brain neurophysiological mechanisms and pathways that control normal grooming behavior (VanErp et al., 1994). It is therefore 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.

ELISA assay demonstrated that administration of cholecalciferol at doses of 1.0 and 2.5 mg/kg in combination with 17β-E2 profoundly increased estradiol and 25-OH-VD3 levels, while the co-administration of cholecalciferol at a dose of 5.0 mg/kg and 17β-E2 in the OVX rats failed to change 25-OH-VD3, but increased estradiol level in the OVX rats after long-term absence of estrogen. It should be noted that synergic antidepressant-like effect of cholecalciferol at dose of 1.0 mg/kg in combination with 17β-E2 associated with the similar synergic effect on the increase of estradiol and 25-OH-VD3 levels in the blood in the OVX rats after long-term absence of estrogen. Although, cholecalciferol at a dose of 2.5 mg/kg plus 17β-E2 induced more profound increase of estradiol and 25-OH-VD3 levels in the OVX rats with long-term absence of estrogen, however, we did not find any modifications of the depression-like behavior in the FST of these rats, because of these rats showed similar depression-like profile like the OVX rats given with 17β-E2 alone. Interestingly, in the present study cholecalciferol exhibited synergic antidepressant-like effect when cholecalciferol was administered at a dose of 1.0 mg/kg SC plus 17β-E2 in the OVX rats with long-term absence of estrogen. In contrast, to our previous study where OVX rats after 2 weeks of post-OVX, the dose of 1.0 mg/kg of cholecalciferol plus 17β-E2 was not effective and we demonstrated synergic antidepressant-like effect of cholecalciferol at dose of 5.0 mg/kg in combination with 17β-E2. In fact, the effects of cholecalciferol at doses of 2.5 and 5.0 mg/kg in a combination with a low dose of 17β-E2 on the depression-like behavior of the OVX rats after long-term absence of estrogen did not associated with its effects on estradiol and 25-OH-VD3 levels in the blood of these OVX rats.

Thus, in the present study, it was observed that at 12 weeks postovariectomy period only a dose of 1.0 mg/kg SC cholecalciferol was effective to reduce depression-like behavior in the FST. In contrast, at 2 weeks post-OVX a more higher dose of cholecalciferol (5.0 mg/kg SC) produced antidepressant-like effect in the FST. Current data suggest that the antidepressant-like effects of cholecalciferol at tested doses are different after 2 weeks post-OVX and 12 weeks post-OVX females. Moreover, 12 weeks post-OVX period significantly alters the antidepressant-like response of treatment with cholecalciferol in OVX rats. Important changes in the endocrine milieu could be expected closer to the removal of ovaries. It is possible that specific sites of action involved in the antidepressant-like effects of cholecalciferol that also modulated by estrogens are affected by the endocrine milieu that prevails at different period for 2 or 12 weeks after surgery. Moreover, after a long-time absence of ovarian fluctuations an adaptive process may contribute to a better response for cholecalciferol administration at a dose of 1.0 mg/kg SC. Thus, the results of this study can be summarized as follows: specific dose of cholecalciferol that was able to induce antidepressant-like effect is dependent from the post-OVX time and hormonal state (intact or OVX rats). Further investigations is to be addressed in relation to such issues. Whether different effects of cholecalciferol in OVX rats follow different time period after surgery, or whether different doses of cholecalciferol in OVX rats with different post-ovariectomy interval might lead to negative versus positive effects. Moreover, further studies are needed to evaluate the association of VD with estrogen-related pathways and to conduct another chronic experiments together with biochemical studies of these subjects to verify the significance of this study.

The role of ovarian hormones in depression and stress sensitivity is of great interest for women transitioning through menopause (Burger, 2008; Maclennan et al., 2004; Vera et al., 2002). Mood disorders during menopause could be partly due to loss of estrogen with menopause because estrogen is known to have neuroprotective effects on brain (Wilkins et al., 2006; Przybelski, Binkley, 2007). Hormone replacement therapy (HRT) may improve the symptoms of depression in depressed people or decrease the risk of developing depressive symptoms in older women, but this is unclear because in some studies HT does not stop the development of depressive symptoms in elderly postmenopausal women (Rossouw et al., 2002; Anderson et al. 2004). The exact role of estrogen still needs to be defined. Menopause are also at higher risk of developing VD deficiency due to decreased dietary intake, less sun exposure, restricted outdoor activity and a decreased capacity to produce enough calcitriol as a result of an age related decline in hydroxylation by kidneys (Cheema et al., 1989; Schnatz et al., 2012; Robbins et al., 2014). VD, a group of steroid compounds, has become of great interest due to many studies which have revealed its role far beyond bone metabolism (Stewart et al., 2010; Kesby et al., 2011; Groves et al., 2014). Through decades VD was considered a vitamin but nowadays it has emerged as an active hormone exerting its action as a transcription factor regulating the expression of numerous genes (Holick, 2006; Penckofer et al., 2010). The presence of VDR outside the skeletal system, enterocytes and renal tubular cells was confirmed in many cell types including immune cells, neurons, pancreatic cells, myocytes, cardiomyocytes, endothelium cells, which stress pleiotropic activity of VD (Holick, 2007). There is a great

 doi:10.20388/omp2018.001.0056

body of evidence confirming that apart from its well-known function in calcium-phosphate homeostasis, VD also exerts many non-calcemic actions in various tissues and systems (Fernandes de Abreu et al., 2009; Kesby et al., 2011; Groves et al., 2014). Vitamin D deficiency has been linked with significant complications such as cardiovascular events, depression, anxiety, cognitive disorders, obesity, metabolic syndrome, type 2 diabetes, various types of cancer, immune disorders (Fernandes de Abreu et al., 2009; Stewart et al., 2010). According to Gaugris and co-workers (2005), the prevalence of low VD levels appears to be high in post-menopausal women. Additionally, the decline of estrogens after menopause decreases the activity of 1α-OHase, what results in lower synthesis of the active VD form (Gaugris et al., 2005; Bikle, 2014). These results suggest that VD supplementation, even in higher doses, may be necessary in postmenopausal women. VD supplementation seems to be the most appropriate treatment option for the population of postmenopausal patients and has been suggested by many experts as a safe and cost-effective procedure. However, the role of VD supplementation in the prevention and treatment of comorbidities associated with menopausal consequences has not been completely established.

Adequate VD status may play a very important role in terms of appropriate brain development and function (Kalueff et al., 2004b; Garsion et al., 2002). Therefore, adequate supply of VD in specific periods of life, including the menopausal period, seems to be of particular importance, because it may reduce the risk of CNS diseases whose treatment is difficult and which represent a heavy burden both for the affected individuals and their society (Eyles et al., 2003). What becomes particularly important in light of these reports is continued study of the effects of VD on CNS function aimed at establishing a recommendation of VD dietary intake, which is a key element in averting its deficiency, and making tests determining serum 25(OH) D concentration generally available in menopausal women (Stewart et al., 2010; Wrzosek et al., 1997). Recent studies have investigated the association between VD and depression; however, the results are conflicting (Carswell, 1997; Kiraly et al., 2006; Penckofer et al., 2010; Studd et al., 2014). These points illustrate how the current state of VD treatment research is incomplete and in need of more intensive research. Working toward uncovering how the interaction between VD and estradiol changes after menopause, and the implications of these changes elsewhere in the post-menopausal woman, is necessary for providing the most complete understanding of how VD treatment alone or in a combination with 17β-estradiol supplementation may affect women's affective-related state.

The female reproductive system is composed of central regulators including the hypothalamus and the pituitary gland and peripheral organs such as the ovary, uterus, and during pregnancy the placenta. VDR expression has been noted throughout the female reproductive tract (Halloran, De Luca, 1980; Kwiecinksi et al., 1989). In vitro studies have shown a direct modulation by vitamin D of estradiol, estrone, and progesterone production in human ovarian cells (Kinuta et al., 2000; Luk et al., 2012; Ozkan et al., 2010). VD as changes in VDR impact on various brain neurotransmitters, and thus suggest a potential role of vitamin D in causing and redressing mood disorders (Puchacz et al., 1996; Kiraly et al., 2006; Groves et al., 2013; Patrick, Ames, 2014). We could suppose, even though estrogens and cholecalciferol share similar targets on monoaminergic or another neurotransmitter systems to induce their antidepressant-like effects, the behavioral effects of cholecalciferol is different in 2 weeks post-OVX and 12 weeks post-OVX females. It is likely that cholecalciferol acts through a different mechanism of action that is sensitive to the long-term absence of ovarian hormones. The physiological function of VD related to the female reproductive system has recently been reported (Avila et al., 2004; Belkacemi et al., 2003; 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 (Nashol 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 downregulates 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 downregulation of Cyp24a1 and upregulation 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.

Interactions between genomic and non-genomic effects of cholecalciferol and 17β-E2 cannot be excluded. It is possible that the time- and concentration-dependent involvement of the non-genomic and nuclear receptor

mediated effects of 17β-E2 might underlie the complex associations between 17β-E2 and cholecalciferol. Taken together these data in can be assumed that of cholecalciferol modulates activity of pituitary-ovary axis. Our future investigations will aim to clarify how chronic cholecalciferol treatment alters functional activity of pituitary-ovary system in female rats with an imbalance of estrogens. Based on our results it may be suggested that cholecalciferol helps to provide significant protection against long-term ovariectomy-induced depression-like behavior. Further studies are needed to be done to understand the detailed mechanism of antidepressant-like effect of cholecalciferol in the OVX rats with long-term absence of estrogen.

Conclusions

The present data of our preclinical study indicates that chronic cholecalciferol at a dose of 1.0 mg/kg SC treatment on depression-related behavior after impairment induced by long-term ovariectomy. The data also indicate that the combination of cholecalciferol at a dose of 1.0 mg/kg SC and 17β-E2 is more effective than 17β-E2 alone in OVX rats inducing a more synergic antidepressant-like effects in the FST. Taken together, it can be proposed that the positive effect of chronic cholecalciferol at a dose of 1.0 mg/kg, SC on depression-related brain function. Furthermore, this is the first study to show a beneficial effect of chronic cholecalciferol at dose of 1.0 mg/kg SC administration on depression-related states induced by long-term ovariectomy in female rats. This work promotes more effective creating of the novel therapeutic targets and strategies for depression treatment in subjects with long-term estrogen deficiency.

Acknowledgments

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

Conflict of Interest

The authors declare no conflict of interest.

References

AMSTERDAM J., GARCIA-EPAÑA F., FAWCETT J., QUITKIN F., REIMHERR F., ROSENBAUM J., BEASLEY C. (1999). Fluoxetine efficacy in menopausal women with and without estrogen replacement. J Affect Disord, 55, 11-17.

ANDERSON G.L., LIMACHER M., ASSAF A.R., BASSFORD T., BERESFORD S.A., BLACK H. (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA, 291, 1701-1712.

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.

BIKLE D.D. (2014). Vitamin D metabolism, mechanism of action and clinical applications. Chemistry and Biology, 21(3), 319-329.

BLOMBERG J.M., NIELSEN J.E., JORGENSEN A., RAJPERT-DE MEYTS E., KRISTENSEN D.M., JOERGENSEN N., SKAKKEBAEK N.E., JUUL A., LEFFERS H. (2010). Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. Hum Reprod (Oxford, England), 25, 1303-1311.

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

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.

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, pp. 1197–1211.

DE CHAVES G., MORETTI M., CASTRO A.A., DAGOSTIN W., DA SILVA G.G., BOECK C.R., QUEVEDO J., CHRISTAKOS S., DELUCA H.F. (2011). Minireview: Vitamin D: Is there a role in extraskeletal health? Endocrinol, 152(8), 2930-2936.

CHEEMA C., GRANT B.F., MARCUS R. (1989). Effects of estrogen on circulating "free" and total 1,25-dihydroxyvitamin D and on the parathyroid-vitamin D axis in postmenopausal women. J Clin Invest, 83, 537-542

DELUCA H.F. (2014). History of the discovery of vitamin D and its active metabolites. Bone Key Rep, 3. 479.

DREVETS W.C., PRICE J.L., FUREY M.L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain structure and function, 213(1-2), 93-118.

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. (2003). Antidepressant-like effect of different estrogenic compounds in the forced swimming test. Neuropsychopharmacol, 28, 830-838.

ESTRADA-CAMARENA E., FERNANDEZ-GUASTI A., LOPEZ-RUBALCAVA C. (2004). Interaction between estrogens and antidepressants in the forced swimming test in rats. Psychopharmacol, 173, 568-601.

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(10), 1365-1377.

EYLES D., BROWN J., MACKAY-SIM A., MCGRATH J., FERON F. (2003). Vitamin D3 and brain development. Neurosci, 118, 641-653.

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

FEDOTOVA J., DUDNICHENKO T., KRUZLIAK P., PUCHANSKAYA Zh. (2016). Different effects of vitamin D hormone treatment on depression-like behavior in the adult ovariectomized female rats. Biomed Pharmacother, 84. P. 1865-1872.

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

HALLORAN B.P., DE LUCA H.F. (1980). Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. J Nutr, 110, 1573-1580.

HEANEY R.P. (2008). Vitamin D in health and disease. Clin J Am Soc Nephrol, 3(5), 1535-1554.

HOLICK M.F. (2006). High prevalence of vitamin D inadequacy and implications for health. Mayo Clinic Proc, 81, 353-373.

HOLICK M.F. (2007).Vitamin D deficiency. New Eng J Med, 357, 266-281.

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.

GAVIOLI E.C. (2009). Effects of long-term ovariectomy on anxiety and behavioral despair in rats. Physiol Behav, 97. 420-425.

GAUGRIS S., HEANEY R.P., BOONEN S., KURTH H., BENTKOVER J.D., SEN S.S. (2005). Vitamin D inadequacy among post-menopausal women: a systemic review. QJM, 98(9), 667-676.

GENARO P.S., PEREIRA G.A.P., PINHEIRO M.M., SZEJNFELD V.L., MARTINI L.A. (2007). Relationship between nutrient intake and vitamin D status in osteoporotic women. Int J Vitam Nutr Res, 77(06), 376-381.

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.

GROVES N.J., MCGRATH J.J., BURNE T.H. (2014). Vitamin D as a neurosteroid affecting the developing and adult brain. Annu Rev Nutr, 34, 117-141.

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.

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

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

KALUEFF A.V., LOUA Y.-R., Laaksib I., Tuohimaaa, P. Increased grooming behavior in mice lacking vitamin D receptors. Physiol. Behav. 2004a, 82, 405-409.

KALUEFF A.V., EREMIN K., TUOHIMAA P. (2004 b). Mechanisms of neuroprotective action of vitamin D3. Biochem, 69, 738-741.

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. (2000). Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinol, 2000, 141, 1317-1324.

KIRALY S.J., KIRALY M.A., HAWE R.D., MAKHANI V. (2006). Vitamin D as neuroactive substance: review. The Scientific World J, 6, 125-139.

KWIECINKSI G.G., PETRIE G.I., DE LUCA H. F. (1989). 1,25-Dihydroxyvitamin D3 restores fertility of vitamin D-deficient female rats. Am J Physiol, 256, E483-E487.

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. Evidence that estrogens modulate activity and increase the number of 1,25-dihydroxyvitamin D receptors in osteoblast-like cells (ROS 17/2.8). Endocrinology 1992, 130, 2597-2601.

LUK J., TORREALDAY S., NEAL PERRY G., PAL L. (2012). Relevance of vitamin D in reproduction. Hum Reprod (Oxford, England, 27, 3015-3027.

MACLENNAN A.H., TAYLOR A.W., WILSON D.H. (2004). Hormone therapy use after the Women's Health Initiative. Climacteric, 7, 138-142.

MAHMOUDI T. (2009). Genetic variation in the vitamin D receptor and polycystic ovary syndrome risk. Fertil Steril, 92, 381-1383.

MAKI P.M., FREEMAN E.W., GREENDALE G.A., HENDERSON V.W., Newhouse P.A., SCHMIDT P.J., SCOTT N.F., SHIVELY C.A., SOARES C.N. (2010). Summary of the National Institute on aging-sponsored conference on depressive symptoms and cognitive complaints in the menopausal transition. Menopause, 17, 815-822.

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 middleaged rats. Pharmaceuticals, 9, 21.

OFFNER H. (2004). Neuroimmunoprotective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: Therapeutic implications for multiple sclerosis. J Neurosci Res, 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, 73, 93-96.

OZKAN S., JINDAL S., GREENSEID K., SHU J., ZEITLIAN G., HICKMON C., PAL L. (2010). Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril, 94, 1314-1319.

PAE C.U., MANDELLI L., KIM T.S., HAN C., MASAND P.S., MARKS D.M., Patkar A.A., STEFFENS D.C., De RONCHI D., SERRETTI A. (2009). Effectiveness of antidepressant treatments in pre-menopausal versus post-menopausal women: A pilot study on differential effects of sex hormones on antidepressant effects. Biomed Pharmacother, 63, 228-235.

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.

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.

PENCKOFER S., KOUBA J., BYRN M., ESTWING FERRANS C. (2010). Vitamin D and depression: where is all the sunshine? Issues Ment Health Nurs, 31, 385-393.

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.

PLUM L.A., DELUCA H.F. (2010). Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov, 9(12). 941-955.

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.

PRZYBELSKI R., BINKLEY N. (2007). Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. Arch Biochem Biophys, 460, 202-220

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.

ROBBINS J.A., Aragaki A., CRANDALL C.J., MANSON J.E., CARBONE L., JACKSON R., LEWIS C.E., JOHNSON K.C., SARTO G., STEFANICK M.L., WACTAWSKI-WENDE J. (2014). Women's health initiative clinical trials: interaction of calcium and vitamin D with hormone therapy. Menopause, 21, 116-123.

ROSSOUW J.E., ANDERSON G.L., PRENTICE R.L., LACROIX A.Z., KOOPERBERG C., STEFANICK M.L., JACKSON R.D., BERESFORD S.A., HOWARD B.V., JOHNSON K.C., KOTCHEN J.M., OCKENE J. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA, 288, 321-333.

SANTORO N., EPPERSON C.N., MATHEWS S.B. (2015). Menopausal Symptoms and their Management. Endocrinol Metab Clin North Am, 44(3), 497-515.

SCHEID V., WARD T., CH W.S., WATANABE K., LIAO X. (2016). The treatment of menopausal symptom, s by traditional East Asian medicines: Review and perspectives. Maturitas, 66, 111-130.

SCHNATZ P.F., MARAKOVITS K.A., O'SULLIVAN D.M., ETHUN K., CLARKSON T.B., APPT S.E. (2012).

Response to an adequate dietary intake of vitamin D3 modulates the effect of estrogen therapy on bone density. J Womens Health (Larchmt), 21, 858-864.

SOARES C.N., ALMEIDA O.P., JOFFE H., COHEN L.S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: A double-blind, randomized, placebo-controlled trial. Arch Gen Psychiatry, 58, 529-534.

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.

STUDD J., NAPPI R.E. (2012). Reproductive depression. Gynecol Endocrinol, 28 (Suppl. S1), 42-45.

THEODORATOU E., TZOULAKI I., ZGAGA L., IOANNIDIS J.P. (2014). Vitamin D and multiple health outcomes: umbrella a review of systematic reviews and meta-analyses of observational studies and randomised trials. BMJ, 348, g2035.

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, restraintand fur moistening. Behav Brain Res, 65, 47-55.

VEDDER L.C., BREDEMANN T.M., MCMAHON L.L. (2014). Estradiol replacement extends the window of opportunity for hippocampal function. Neurobiol Aging, 35, 2183-2192.

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-dihydroxy-vitamin D3 receptor immunoreactivity in the limbic system. Horm Metab Res, 33, 525-531.

WRZOSEK M., LUKASZKIEWICZ J., JAKUBCZYK A., MATSUMOTO C., 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, 1197-1211.

WILKINS C., SHELINE Y., ROE C., BIRGE S., MORRIS J. (2006). Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. Am J Geriatr Psychiatry, 14, 1032-1040.

ZARINANI A.H., SHAHBAZI M., SALEK-MOGHADDAM A., ZAREIE M., TAVAKOLI M., GHASEMI J., REZANIA S., MORAVEJ A., TORKABADI E., RABBANI H., JEDDI-TEHRANI M. (2010) Vitamin D3 receptor is expressed in the endometrium of cycling mice throughout the estrous cycle. Fertil Steril, 93, 2738-2743.