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Mammalian target of rapamycin (mTOR)/nitric oxide system possibly modulate antidepressant-like effect of 17α-ethinyl estradiol in ovariectomized mice

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ABSTRACT

Due to a close association between depressive disorders and altered estrogen levels, this study was conducted to examine the hypothesis that antidepressant-like effect of ethinyl estradiol (EE_2) in ovariectomized mice is modulated by mammalian target of rapamycin (mTOR)/nitric oxide pathways.

Female mice were undergone bilateral ovariectomy and different doses of EE_2 were intraperitoenally injected alone and combined with specific mTOR inhibitor, rapamycin, non-specific NOS inhibitor, L-NAME, nNOS inhibitor, 7-NI, NO precursor, L-arginine, and selective PDE5I, sildenafil. After locomotion assessment, immobility times were recorded in FST and TST. Moreover, hippocampal mTOR expression was assessed using western blot assay. The hippocampal concentrations of nitrite, a major metabolite of NO, were measured.

Although EE_2 demonstrated a significant antidepressant-like activity in OVX mice, acute rapamycin exerted an unmarked decrease of the anti-immobility effect of EE_2 in FST and TST ($P > 0.05$). In contrast, combination of minimal effective dose of EE_2 with sub- effective doses of either L-NAME (10 mg/kg) or 7-NI (25 mg/kg) resulted in a robust antidepressant-like effect in OVX mice. Administration of either L-NAME or 7-NI enhanced the decreased antidepressant activity of EE_2 induced by combination with rapamycin. Moreover, decrement of hippocampal mTOR expression in OVX mice was significantly enhanced by acute EE_2. The increased hippocampal nitrite concentrations caused by ovariectomy were also reversed by EE_2 administration.

The study demonstrated that acute treatment with lowest dose of EE_2 exerts significant antidepressant-like behavior in OVX mice, possibly, through mTOR activation. This effect seems to be also mediated by the suppression of nitric oxide pathway.

1. Introduction

There is growing body of evidence that depressive disorders with nearly two-fold more prevalence in women can be associated with fluctuation of sexual hormones, particularly, estrogens [1,2]. Interestingly, depressive disorders are 4-fold more prevalent in post-menopausal women rather than the women before menopause. Hence, decreased levels of estrogens during pre- and post-menopause, ovariectomy, postpartum and premenstrual dysphoric disorders are consistent with episodes of lowered energy, activity and mood [3–5]. This condition can be relieved by estrogen replacement therapy, whereas recent studies have shown that long-term treatment with 17β-estradiol or conjugated equine

Abbreviations: OVX, ovariectomized; EE_2, 17α-ethinyl estradiol; mTOR, gamma Mammalian target of rapamycin; NO, Nitric oxide; cGMP, cyclic GMP; L-NAME, N^{ω}-nitro-L-arginine methyl ester; 7-NI, 7-nitroindazole; NOS, Nitric oxide synthase; eNOS, endothelial NOS; nNOS, neuronal NOS; PDE5I, phosphodiesterase type 5 inhibitor; FST, Forced swimming test; TST, tail suspension test; OFT, open-field test.

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estrogens significantly attenuate depression symptoms during postmenopausal and postpartum conditions [2]. In addition to clinical studies, pre-clinical investigations have confirmed the antidepressant-like effect of 17β-estradiol and 17α-ethinyl estradiol in bilaterally ovariectomized mice in the forced swimming and tail suspension tests [2,4].

Although there are some identified aspects of estrogens activity modulated by monoaminergic and nitrergic systems in different regions of brain, the underlying mechanisms of estrogens in affective disorders are not obviously understood. Previous studies have reported that 17β-estradiol exerts the neuroprotective effects in cerebral ischemic injury and neural defects through activation of Phosphoinositide-3-kinase (PI3 K)/Akt signaling pathway [6]. Moreover, they demonstrated a major role for phosphorylated form of downstream targets of Akt, mTOR/p70S6 K signaling cascades, in the antidepressant-activity revealed by 17β-estradiol [7]. The fact is strengthen by a post-mortem study that deficits in mTOR signaling pathway were found in the prefrontal cortex of patients with depression [8,9].

The mammalian target of rapamycin (mTOR), a serine/threonine kinase component downstream of PI3 K/Akt signaling pathway, plays a key role in mRNA translation, cell growth and endocrine resistance [10]. Clinical and biological evidences suggested that dysregulation of mTOR pathway contributes with a variety of human diseases such as depression [11–13]. It has been shown that sub-chronic, but not acute, treatment with rapamycin, a mTOR inhibitor, exerts a significant antidepressant-like effect in mice. In addition, Moretti et al. (2014) have indicated that activation of PI3 K and mTOR are defined as essential mechanisms underlying antidepressant-like activity of ascorbic acid [14]. These findings can provide novel pharmacological target for interpretation of antidepressant activity of current antidepressant drugs and for future discovery of pathophysiology of depression and the development of antidepressants.

On the other hand, it has been suggested that NOS inhibitors can be used as antidepressants through reducing cyclic guanosine monophosphate (cGMP) and NO levels, especially, following bilateral ovariectomy in female mice [15–18]. NO is biosynthesized from L-arginine by three types of nitric oxide synthase (nNOS, iNOS and eNOS) [19–21] and exerts its biological function via its downstream effector cGMP [22]. Some reports found that rapid modifications occurring in nitrergic pathway of female hippocampus during ovarian cycle may suggest it as a target for estrogen-mediated actions [23]. In addition, it is observed that estradiol can exert its antidepressant-like effect via inhibition of nNOS in ovariectomized (OVX) mice [4].

There is no information regarding the involvement of mTOR/NO signaling pathways in antidepressant activity of estrogens following ovariectomy. Therefore, the current study aimed to investigate the hypothesis that acute 17α-ethinyl estradiol (EE_2) has antidepressant-like activity in OVX mice. In addition, this study was conducted to examine the assumption that the antidepressant-like activity of EE_2in the ovariectomy-induced depressive mice is modulated by mTOR and L-arginine–NO–cGMP pathway.

2. Materials and methods

2.1. Animals

Female NMRI mice weighing 20–30 g were used and kept in 12-h regular light/dark cycle (lights on at 06:00–18:00) at temperature of 21–23 °C. Animals had free access to food and water except for the brief time of ovariectomy and the behavioral tests (open-field, forced swimming and tail suspension tests).All behavioral tests were performed between 12:00 to 18:00 h. The mice were placed into three main groups including un-operated, sham-operated and OVX mice receiving saline and drugs. Each animal was undergone the operation and behavioral tests only once and the number of animals in each studied group was 6–8. All examinations were performed in accordance to the NIH Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and based on the guidelines of institutional animal care and use committee (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences). The experiment was approved by Ethics Committee of Tehran University of Medical Sciences.

2.2. Ovariectomy

The surgical procedure was conducted under a general anesthesia with ketamine HCL (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). The lumbar dorsum zone was shaved and prepared for aseptic surgery (a 10% povidone-iodine scrub followed by a sterile saline wipe). The ovariectomy was performed as previously described [24,25]. Briefly, parovarian fatty tissue was identified and pulled out. The exposed ovary and associated oviduct were removed. Ultimately, skin and muscles were sutured (6-0 non-absorbable). In sham-operated animals, the parovarian fatty tissues and ovaries were only retracted and replaced. All behavioral studies were initiated 10 days after the ovariectomy [4,26].

2.3. Behavioral studies

2.3.1. Open-field test

Mice were subjected to the open-field test prior to the FST and TST, in order to rule out a possible interference of the locomotion in the interpretation of the data resulted from the depression despair animal tests [27–29]. Open-field apparatus is a Plexiglas box with dimensions of 40 cm × 60 cm × 50 cm. The floor is divided into 12 equal squares. The animals were adapted to the experimental room for at least 1 h before the test, and were placed gently in the left corner of the field and, finally, the number of square crosses with all paws was counted in a 6-min period. Dim light was provided inside the apparatus to avoid anxiety behavior. After each test the apparatus was cleaned with a solution of 10% ethanol to hide animal clues.

2.3.2. Forced swimming test

The mice were individually placed in an open vertical Cylindrical glass container (diameter: 10 cm, height: 25 cm) containing water up to 19 cm at temperature of 23 ± 1 °C. After open-field test, the mice were allowed to swim based on the procedure involved a 6-min test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in water, making only those movements necessary to keep its head above water. The duration of immobility was recorded during the last 4 min of the test [30]. Whole 6-min test were video-taped for each mouse and immobility times were calculated by an observer unaware of the animal groups. After the test, mice were dried with towels and transferred to a drying cage situated under a warming lamp (30 ± 1 °C).

2.3.3. Tail suspension test

The duration of immobility caused by tail suspension was recorded during a 6 min test in accordance to the method established by Steru et al. [31]. In brief, the acoustically and visually isolated mice were suspended from their tail using adhesive tape 50 cm above the floor. Whole 6-min test were video-taped for each mouse and immobility times were calculated by an observer unaware of the animal groups.