Life Sciences 84 (2009) 512–516

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Subchronic stress-induced depressive behavior in ovariectomized mice

Osamu Nakagawasai [a,*], Akira Oba [a], Atsushi Sato [a], Yuichiro Arai [b], Satoru Mitazaki [c], Hiroshi Onogi [a], Kenji Wakui [a], Fukie Niijima [a], Koichi Tan-No [a], Takeshi Tadano [a]

[a] Department of Pharmacology, Tohoku Pharmaceutical University, Sendai 981-8558, Japan
[b] Division of Pharmacology, School of Pharmaceutical Sciences, Ohu University, Koriyama 963-8611, Japan
[c] Laboratoy of Forensic Toxicology, Faculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki 370-0033, Japan

ARTICLE INFO

Article history:
Received 7 October 2008
Accepted 26 January 2009

Keywords:
Depression
Estradiol
Forced swimming test
Ovariectomy
Stress

ABSTRACT

Aims: Mood disorders including depression are more common in women than men, particularly in times of lower estradiol levels. In this study, we investigated the effect of estrogen on emotional behavior in mice in a stress environment.
Main methods: Female mice were divided into four groups: two groups were ovariectomized (OVX) and two were sham-operated. One group each of OVX and sham mice was kept in a normal environment and the other groups were assigned to a daily stress (1 h/day) for 7 days from 5 days after operation. On the 14th day after operation, subjects were measured to assess behavioral specificity, locomotor activity, elevated plus-maze (EPM) behavior, passive avoidance (PA) behavior and forced swimming behavior.
Key findings: The OVX plus stress (OVX + S) group showed a significant prolongation of immobility compared with the other groups. In all the groups there were no changes in locomotor activity, EPM behavior or PA behavior. We further examined the effect of estrogen against depressive behavior in the OVX + S group. The vehicle or 17β-estradiol (E2) was administered s.c. to OVX + S mice for 4 days beginning on post-operative day 11. Subchronic E2 treatment decreased the stress response and improved depressive behavior relative to the vehicle group.
Significance: These data have important implications regarding the prevention of depression in postmenopausal women undergoing estrogen therapy.

Introduction

There are sex differences in vulnerability for affective disorders, such that prevalence rates for anxiety disorders and major depression among women are approximately twice that seen in men (Seeman 1997). Numerous clinical studies implicate the importance of estrogen in a number of hormone-related affective disorders in women, such as premenstrual dysphoric symptoms, postpartum depression, and peri- and post-menopausal depression (Rubinow and Schmidt 1995; Wittchen and Hoyer 2001; Young et al. 2000). Estrogen replacement effectively augmented the effects of selective serotonin re-uptake inhibitors in women suffering from therapy resistant major depression (Rasgon et al. 2002). Ovariectomized (OVX) female rats treated with estrogen display a decrease in anxiety-related behavior in repeated open field exposure and reduced immobility in the forced swimming test (Bowman et al. 2002; Rachman et al. 1998). These data suggest that estrogen is involved in the modulation of mood and emotion. Although there is growing evidence to support the hypothesis that ovarian hormone loss plays a role in the depression of menopausal women, postmenopausal women do not always suffer from depression. In a preliminary study, we found that OVX mice did not show some abnormal symptoms such as cognitive deficit, anxiety and depression. It has been reported that hormone loss during menopause is not associated with an increased incidence of depression (for review, see Wollersheim 1993). Other events, including psychosocial factors such as stress (e.g., death of partner or child after menopause), are associated with higher risks of depression in women (Kaufert et al. 1992; Hunter 1992; Avis and McKinlay 1991). Epidemiological studies also show that stressful life experiences are associated with the onset of affective disorders, like major depression and anxiety disorders (Kendler et al. 1995; Post 1992). It has been reported that brain activity in the limbic system and hippocampal areas of rats is affected by chronic stress exposure (Kaufman et al. 2000; McEwen 2000; Trentani et al. 2002). Accordingly, chronic stress exposure is used as an animal model for studying the development and treatment of affective disorders.

In this study, to clarify the involvement of stress on postmenopausal depression and to develop a reliable animal model of the disease, we investigated whether some behaviors such as locomotor activity, duration of open arm on the elevated plus-maze, latency time on the passive avoidance task and duration of immobility on the

* Corresponding author. Department of Pharmacology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan. Tel./fax: +81 22 727 0123.
E-mail address: osamun@tohoku-pharm.ac.jp (O. Nakagawasai).

0024-3205/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2009.01.009

forced swimming test were altered following chronic stress-subjected ovariectomy, and whether subchronic administration of estradiol reversed the effects of the ovariectomy.

Materials and methods

All experiments were performed according to the Guide for Care and Use of Laboratory Animals at Tohoku Pharmaceutical University.

Experimental animals and stress exposure

Female ddY mice, 8 weeks of age, were obtained from Japan SLC (Hamamatsu, Japan) and acclimated for 3 days. The mice were maintained under conditions of constant temperature (23 ± 1 °C) and humidity (55 ± 5%) on a 12 h/12 h light-dark cycle (light from 9 to 21 h; dark from 21 to 9 h). The mice had free access to food and water throughout the experimental period. They were housed in plastic cages (31 cm × 21 cm × 13 cm) and divided into four different groups as follows: sham with no stress group (Sham-S, $n = 36$), sham with chronic stress group (Sham + S, $n = 65$), ovariectomized with no stress group (OVX-S, $n = 27$) and OVX with chronic stress group (OVX + S, $n = 59$). OVX was performed by removing the bilateral ovaries under ether anesthesia. Success of the operation was confirmed by demonstration of predominantly leukocytes with few epithelial cells in vaginal smears from the 2nd to the 5th day after OVX. We have used sham including all estrous cycle mice.

The procedure of stress exposure with some modifications was performed as previously described (Mizoguchi et al. 2000). Briefly, the animals were placed in a plastic cylinder (2.5 cm internal diameter, 10 cm length), the front end of which was closed by means of a wire net and immersed to the level of the xiphoid process in a water bath (21 °C) for 1 h. The animals were subjected to this stress session once a day for 7 days from the 5th to the 11th day after OVX. The behavioral tests were performed on the 14th day after OVX.

Drugs

17 β-estradiol (E2; Sigma, St-Louis, USA) was dissolved in sesame oil (Wako Pure Chemical Industries Ltd, Osaka, Japan) and daily treated by s.c. injection at two doses (vehicle and 0.1 mg/kg/day) from the 11th to the 14th day after OVX surgery.

Measurement of locomotor activity

The locomotor activity in OVX mice were evaluated using Animex Auto MK-110 (Muromachi Kikai Co., Ltd., Tokyo, Japan) on the 14th day after OVX. Every movement of the mice, which were placed on the top of the Animex, produced a signal due to variation in inductance and capacity of the apparatus resonance circuit. Then the signals were automatically converted to numbers. On the day of the experiment the mice were individually placed in activity cages and were allowed 15 min to adapt to the new environment. Following adaptation, the number of activity counts was recorded for the total locomotor activity over a 90 min period.

Forced swimming test

Measurement of the immobility in mice was carried out according to a modification to the method of Porsolt et al. (1978). Mice were individually placed in vertical glass cylinders (height 16 cm; diameter 10 cm) containing 8 cm of water maintained at 25 °C, and the total duration of struggle behavior, swimming and immobility were measured during a 5 min test. A mouse was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line. Swimming was scored when the mouse made active swimming motions, i.e. moved around in the cylinder in a horizontal paddling position. Struggle behavior was scored when the mouse was making active movements with its forepaws in and out of the water; these were usually intense movements directed against the wall. The forced swimming test was performed on the 14th day after OVX.

Elevated plus-maze

The elevated plus-maze used in this study was constructed in this laboratory using a modification of the original apparatus for mice, as described in a previous report (Kuribara et al. 2000). Briefly, the plus-maze, which was maintained at a 40 cm elevation, consisted of four arms (6 cm × 30 cm each) that extended from a central platform (9 cm × 9 cm). Two of the arms had 10 cm high sidewalls. Like the central platform, both the floor and sidewalls of these arms were nontransparent and painted gray. The other two arms had no sidewalls, and the floor was constructed of transparent acrylic. For testing, each mouse was placed on the central platform, randomly facing one of the enclosed arms. The cumulative time spent in one of the open arms during the ensuing 5 min period was recorded by a trained observer who was not apprised of the treatment code for the individual groups. A mouse was considered to have entered an open arm if all four paws crossed the border between the central platform and the open arm.

Step-through passive-avoidance task

Training and retention trials for the passive-avoidance task were conducted in a Plexiglas box that contained dark (25 cm × 25 cm × 25 cm) and light (14 cm × 10 cm × 25 cm) compartments. The floor was constructed of stainless steel rods. The floor rods in the dark compartment were connected to an electronic stimulator (Nihon Kohden, Tokyo, Japan). The mouse was placed in the light compartment of the box. As soon as the mouse completely entered the dark compartment, an electric shock (1 mA for 500 ms) was supplied to the floor bars. All mice were exposed to the electric shock once before starting the surgery. As a retention trial, on the 14th day after the surgery, each mouse was placed in the light compartment again for a cut-off time of 600 s. Latency time was estimated by measuring the length of time for the mouse to move from the light to the dark compartment.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). The significance of differences was determined by the Student's *t*-test for two-group comparison, and by a one-way analysis of variance (ANOVA), followed by Fisher's PLSD test for multigroup comparisons, respectively. $p < 0.05$ represented a significant difference.

Results

Locomotor activity

Fig. 1 shows the effect of ovariectomy and/or stress on locomotion. On the 14th day after surgery, total locomotor activity over the 90 min was not significantly different between the groups [$F(3, 37) = 1.12$, $p > 0.05$; Fig. 1]. Although, the locomotor activity in Sham-S group trends to increase, no significant differences between other groups were observed.

Effect of ovariectomy and stress on the forced swimming test

Fig. 2 shows the effect of ovariectomy and/or stress on struggle (A), swimming (B) and immobility (C) behaviors. The duration of struggle behavior in OVX + S group was significantly decreased