Pharmacological Reports 68 (2016) 109–115

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Hypoestrogenism alters mood: Ketamine reverses depressive-like behavior induced by ovariectomy in rats

Sonia Fatima da Silva Moreira [a,c,d], Ellen A. Nunes [a,e], Jonnsin Kuo [a], Isabel Cristina de Macedo [a,e], Alexis Muchale [a], Carla de Oliveira [a,c], Vanessa L. Scarabelot [a,e], Paulo Ricardo Marques Filho [a,c], Liciane F. Medeiros [a,e], Wolnei Caumo [a,c], Iraci L.S. Torres [a,b,c,*]

[a] Pharmacology of Pain and Neuromodulation Laboratory: Animals Models, Department of Pharmacology, Universidade Federal do Rio Grande do Sul Institute of Basic Health Sciences, Porto Alegre, Brazil
[b] Animal Experimentation Unit and Graduate Research Group, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[c] Graduate Program in Medical Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[d] Faculdade de Medicina, Instituto de Ciências da Saúde, Universidade Federal do Pará, Belém, Brazil
[e] Graduate Program in Biological Sciences: Physiology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 19 June 2015
Accepted 23 June 2015
Available online 8 July 2015

Keywords:
Menopause
Depression
Ketamine
Female rats
Aging

ABSTRACT

Background: Estrogen deficiency is associated with the onset of depressive and anxiety symptoms, cognitive impairment, and adverse consequences. We investigated depressive-like behaviors in ovariectomized rats and ketamine's effect on this behavior.
Methods: Twenty-eight female Wistar adult rats were initially divided into two groups: ovariectomized (OVX) and sham surgery (SHAM). Hormonal status was verified by vaginal cytology, and the rats were subjected to a forced swimming (FS) test 18 days post-surgery, an open field (OF) test 28 days post-surgery, and an elevated plus maze (EPM) test 38 days post-surgery (Experiment 1). In addition, the effect of ketamine on depressive-like behavior of the female rats was evaluated (Experiment 2).
Results: OVX group exhibited anxiety-like behavior on EPM test (lower time spent and fewer entries in the open arms), without any difference in performance in the OF test. OVX rats showed depressive-like behavior (higher time of immobility) than SHAM rats in FS test. The SHAM group showed signs of hypoestrogenism (anestrus) at six months of age. Moreover, ketamine was able to reverse depressive-like behavior in the FS retest in both groups (OVX and SHAM).
Conclusion: Similar to the literature, we showed the antidepressant effect of ketamine in depressive female rats which was induced by ovariectomy; including in female rats submitted to sham surgery that interestingly presented a premature menopausal.

Introduction

Menopause is a physiological process caused by the loss of ovarian follicular activity leading to a decrease in the production of estrogens. Hypoestrogenism can cause a variety of physiological and psychological disorders such as changes in the menstrual cycle, vasomotor and genital symptoms, sleep problems, mood swings, and impaired cognitive function [1,2]. Age, menopausal status, chronic diseases and socio-demographic characteristics (income, ethnicity and educational level) have been identified as predictors of the frequency and severity of menopausal symptoms [2,3].

Data from two cohort studies in the United States showed increased risk of depression in women who enter menopausal transition [4,5]. However, the mechanisms responsible for the development of depression in perimenopausal women remain unclear [6,7]. On the other hand, Díaz-Véliz et al. suggest that ovarian hormones modulate anxiety levels and cognitive functions [8]. Anxiety may be a precursor for depression development [9] or may be accompanied by symptoms of depression [10], thus, it is important to consider equally anxiety and depression symptoms when investigating factors that may affect mood, such as hormonal status [11,12].

* Corresponding author.
E-mail address: iracitorres@gmail.com (Iraci L.S. Torres).

http://dx.doi.org/10.1016/j.pharep.2015.06.009
1734-1140/

Depressive and anxious symptoms can significantly reduce the quality of life of postmenopausal women [13]. The mechanisms that lead to the emergence of these symptoms in the menopausal transition are not well understood, and even the pathophysiology of depressive disorders has been widely debated. The monoamine theory posits that depression is caused by a decreased function of monoamines in the brain, and antidepressant drugs are designed to increase the supply of these substances by inhibiting reuptake (serotonin and norepinephrine reuptake inhibitors) or degradation (monoamine oxidase inhibitors) [14]. However, the long time to the onset of the therapeutic action and the low rates of remission has encouraged the search for more effective drugs. The observation that small doses of ketamine produce a rapid and transient antidepressant effect increased the interest in neurobiological systems that were not explored in depression [15]. Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist used in human and veterinary medicine, primarily for the induction and maintenance of general anesthesia. Berman et al. published the first report of ketamine's therapeutic effects on major depressive disorder [15]. After this randomized controlled trial (RCT), many studies provided evidence that a single, intravenous (*iv*), subanesthetic dose of ketamine may relieve depressive symptoms within hours [16]. Garcia et al. suggest that the increase of hippocampal brain-derived neurotrophic factor (BDNF) protein levels induced by ketamine might be necessary to produce a rapid onset of antidepressant action [17]. Another study from the same laboratory demonstrated that Ketamine alters behavior and decreases BDNF levels in the rat brain as a function of time after drug administration, suggesting that the effects of ketamine on behavior and BDNF levels are related to the time at which they were evaluated after administration of the drug [18].

To understand the pathophysiology of anxiety and depression disorders associated with the decline of endogenous estrogen levels and devise interventions aimed at attenuating these symptoms, it is very important to establish and study animal models of menopause. This study investigated depressive-like and anxiety-like behaviors and cognitive performance in ovariectomized rats and ketamine's effect on performance on a FS test.

Materials and methods

Animals

Twenty-eight female Wistar rats (90 days old, 200–300 g) were randomized by weight and housed in cages of polypropylene material (49 cm × 34 cm × 16 cm). They were housed four per cage and maintained with food and water available ad libitum on a 12 h light/dark cycle (lights on at 7:00 AM, and lights off at 7.00 PM) in a temperature-controlled environment (22 ± 2 °C). The animals were initially distributed into two groups: ovariectomized (OVX) and false surgery (SHAM) and subjected to the FS at 18th day post-surgery, open field test (OF) at 28th day post-surgery, and elevated plus maze test (EPM) at 38th day post-surgery. When the animals reached 180 days old, each group was subdivided into two more groups, which received ketamine or vehicle and were again subjected to the forced swimming test. The rats were handled for seven days prior to the experiments and remained in the laboratory for at least 30 min before being submitted to each test. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol No. 110586) and were compliant with Brazilian guidelines involving the use of animals in research (Law N°. 11.794). Additionally, all efforts were made to minimize suffering, pain and discomfort of the animals, as well as to reduce the number of animals.

Surgical procedures

One set of Wistar female rats underwent ovariectomy (surgical removal of the ovaries) and the other set underwent SHAM surgery (opening of the abdominal cavity and sewing it back). At 90 days of age, the rats were anesthetized with ketamine (80 mg/kg, *ip*; Syntec, Brazil) and Xylazine (20 mg/kg, *ip*; Sespo, Brazil) and underwent bilateral ovariectomy. The surgery consisted of a transversal dorsolateral incision of skin, between the last rib and pelvis and muscle dissection in order to expose the abdominal cavity. The ovary is 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 ligated, and the ovary was removed by cutting above the clamped area. The muscle and the skin incision were closed with poligalactin and nylon suture. This procedure was repeated at the other side for bilateral ovariectomy. In SHAM surgery, rats underwent the same incisions, the ovaries and fallopian tubes were exposed and then put back in the abdominal cavity and the muscle and skin were closed. To reduce pain, all rats received dipyrone (25 mg/kg *ip*) after surgery, and recovered for 10 days.

Vaginal smear

Ten days after surgery, vaginal smear was daily obtained in both groups to verify hormonal status. Samples were obtained and analyzed as described by Goldman et al. [19].

Behavioral tests

Locomotor activity assessed by OF test

The behavioral assessment was performed in a varnished wood cage, measuring 60 cm × 40 cm × 50 cm with a glass front wall. The floor was covered with linoleum and divided up with dark lines: 12 squares of 13 cm × 13 cm each. The rats were gently placed in the left back corner and allowed to explore the surroundings for 5 min. The number of line crossings was taken as a measure of locomotor activity [20]. Rearing was defined as the moment the rat rose up on its hind legs, ending when one or both front paws touched the floor again [21], being evaluated as exploratory activity [22]. Grooming was defined as licking/washing of the head and body; it was assessed as a biological function of caring for the surface of the body [23]. The start of a trial occurred immediately after the rat was placed in the environment for scoring purposes. In this test, the animal was recorded as entering a new area when all four paws crossed the boundary into a different, marked-out area. Five measures were taken during the five-min test sessions: latency to leave the first quadrant (time in seconds); number of line crossings (i.e. horizontal activity), outer and inner crossings; number of rearing behaviors (i.e. vertical activity); grooming (time in seconds); and number of fecal boluses. The box was thoroughly cleaned using 70% alcohol between each trial.

Anxiety-like behavior assessed by elevated plus-maze (EPM) test

The EPM test was used to evaluate anxiety-like behavioral state. The maze was made of black PVC and elevated to a height of 50 cm above floor level. The apparatus included two open arms and two closed arms (50 cm × 40 cm × 10 cm), which extended from a common central platform (10 cm × 10 cm). The animal was placed in the central area of the EPM, facing one of the closed arms. Next, the following behavioral measures were recorded during the five-min test sessions: number of protected head-dipping movements (PHD); number of non-protected head-dipping movements (NPHD); number of entries in the open arms (EOA); number of entries in the closed arms (ECA); time spent on the open arms