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Brief communication

Sex-dependent antidepressant effects of lower doses of progesterone in rats

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ABSTRACT

Major depression is more prevalent among women than men, and progesterone might be involved in the mechanisms that generate these differences. Progesterone is clinically used for women in several reproductive events, but its antidepressant effect is unclear. Animal studies showed the interference of progesterone on depressive behaviors of rodents, but they are inconclusive, and no study compared different treatment durations. This study investigated the antidepressant effect of low doses of progesterone in male and female rats under acute or chronic administration. Male and female Wistar rats in different phases of the estrous cycle were acutely administered different doses of progesterone (0.0, 0.4. 0.8 and 1.2 mg/kg) and tested in the forced swimming test (FST). The lowest dose of progesterone (0.4 mg/kg) was chronically administered during two complete estrous cycles and diestrous II female and male rats were tested in the FST. Progesterone decreased depressive-like behaviors only in chronically treated diestrous II female rats and increased immobility in male rats. This low dose of progesterone did not interfere in the hormonal cycling in female rats. Results also showed that diestrous II female rats had greater immobility than male rats in the FST. The greater immobility of diestrous II female rats shows that rats in this estrous phase present more depressive-like behaviors that may be associated with their lower serum levels of progesterone. We showed that progesterone chronically administered at low doses reverses these depressive-like behaviors and has an antidepressant effect during the diestrous II phase of the estrous cycle.

1. Introduction

Major depression is a disorder that has a higher prevalence among women than men [1,2]. This higher prevalence is more evident during the reproductive years, and gonadal hormones might be at least partly responsible for this difference [3,4]. In fact, depression in women seems to be associated with a sudden change in progesterone plasma levels. Studies have shown that the progesterone metabolism to the 3α,5α-reduced allopregnanolone is required for progestins to have an antidepressant effect on women and rodents [5–9]. Some studies suggest that women suffering from depression during the postpartum or peri-menopause period fail in converting progesterone to allopregnanolone, or have an irregular response to naturally occurring decreases in central GABA levels combined with decreased levels of allopregnanolone [10–12]. Thus, affective disorders in women during the menstrual cycle, after delivery and during the menopausal transition could be directly or indirectly associated with progesterone fluctuations.

The forced swimming test (FST) is a predictive assay of depressive behavior in rodents [13]. The validity of the FST is supported by evidence that classical and nonclassical antidepressants significantly reduce depressive-like behaviors in rodents [9,14,15]. Also in rodents, gonadal hormones may influence depressive behavior [16,17]. Changes in endogenous progesterone levels and their association with depressive-like behaviors have been studied in female rodents across the estrous cycle, pregnancy, lactation and postpartum condition [7,18,19]. During proestrous, when levels of progesterone are elevated, female rats have less immobility than male or female rats in other stages of the estrous cycle in the FST [7].

Similarly, pregnant rats have fewer depressive behaviors than postpartum female rats, and hormone withdrawal increases depressive-like behaviors in female rats [18,20]. However, contradictory effects of systemic progesterone administration to male and female rodents have been observed in the FST. Although higher doses of progesterone (10 mg/kg) have been shown to increase or not affect immobility in the FST [21], lower doses (0.5–2.0 mg/kg) seem to decrease depressive-like behaviors in rodents in the FST [6,22]. Additionally, these different behavioral responses also may be related to differences in response to acute and chronic stress [16]. It has been shown that depressive behavior in the FST is sensitive to modification by chronic antidepressant treatments at low doses that were ineffective after acute treatment [16,24,25]. These results suggest that progesterone affects the depressive behavior of male and female rodents, but are inconclusive, and no studies have compared different treatment regimens. This study investigated the antidepressant effect of progesterone in male and female rats under acute or chronic administration using the lowest progesterone dose able to produce

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behavioral effect without altering hypothalamus–pituitary–gonadal axis.

2. Materials and methods

2.1. Animals

Experiments were conducted with adult female and male Wistar rats weighing 250–350 g, housed in groups of four in polypropylene cages (40×33×17 cm) under standard environmental conditions, such as room temperature of 22±2 °C, 12 light–dark cycles with lights on at 7:00 pm, and free access to food and water. The animals were bred and raised in the animal facility of Universidade Federal de Ciências da Saúde de Porto Alegre — Brazil (UFCSPA). Experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and in accordance with the Brazilian Law for the Scientific Use of Animals. Study protocols were approved by the Ethics Committee for Experimental Procedures of UFCSPA, Porto Alegre, Brazil.

2.2. Drugs

Progesterone (4-Pregnene-3,20-dione, Sigma, St. Louis, MO, USA) was dispersed in 0.1% Tween 80 and then dissolved in saline solution. The control group received an equal volume of vehicle only. In the acute treatment performed to determine the dose–response curve from progesterone, male and female rats received IP injections of vehicle or progesterone at 0.4, 0.8 or 1.2 mg/kg. These doses were selected considering the lower doses reported in the current literature with effect on depressive behavior of rodents [6,19–22,33]. In the chronic treatment, an IP dose of progesterone was administered daily, for 8 to 10 days, during two complete estrous cycles. To chronic treatment, the lowest dose of acute treatment was chosen (0.4 mg/kg) in order to verify its depressant effect and to avoid possible interference over estrous cycle — commonly reported in progesterone administration [6,19–22,33].

2.3. Estrous cycle

The estrous cycle for all female rats was determined by daily vaginal smears for at least 14 days. Only females showing two regular 4–5 day cycles were used [16]. Female rats in proestrous or diestrous II were selected for the acute experiment, and females in diestrous II, for the chronic experiment. To determine whether chronic progesterone treatment would affect the estrous cycle of the female rats, vaginal smears were performed throughout the treatments.

2.4. Forced swimming test (FST)

The rats were submitted to the FST according to a protocol slightly modified from the one originally described by Porsolt et al. [13]. Briefly, the rats underwent two trials in which they were forced to swim in an inescapable pool (22×22×35 cm) filled with 27 cm of cool water (25 °C). In the acute treatment study, a 5-min re-test was conducted 24 h after the initial 15-min test [13]. Three drug doses were administered 24, 5 and 1 h before the FST re-test, according to Porsolt's protocol [13]. In the chronic treatment study, male and female rats received doses of 0.4 mg/kg of progesterone or vehicle daily.

The experiments were recorded with a videocassette camera for later ethological analysis of the duration of the acts and postures during the 5-min FST re-test. After each swimming session, the animals were gently towel-dried and returned to their cages. To rule out pharmacological effects on general motor activity that might account for behavioral patterns in the FST, the rats were placed in an automated locomotion apparatus (Albarsch, Porto Alegre, Brazil) immediately before the FST re-test session for 5 min to assess their locomotor activity, as proposed by Porsolt et al. [13] and previously described [16]. The swimming sessions were always conducted between 1 and 3 pm and lasted for 15 min to minimize the effect of circadian rhythms on behavioral results.

2.5. Behavioral analysis

Behavioral analyses were conducted by a trained researcher blinded to the different treatment protocols. The videotapes were analyzed by direct computer keyboard input into Basic-written software, as already described [17,23]. Immobility was defined as the total cessation of all movements except breathing. Additionally, two measures of activity were assessed: climbing and swimming duration. Climbing was defined as moving the forepaws up and down against the pool wall, and swimming was defined as vigorous lateral movements of the forepaws while facing away from the pool wall and moving a distance of at least 15 cm or moving to keep the head above water. The presence of depressive-like activity was inferred from a significant amount of immobility time and a reduction in the amount of time spent in active behaviors, such as climbing or swimming.

2.6. Statistical analysis

The statistical analysis of the acute and chronic experiments was performed using a two-way analysis of variance (2-way ANOVA) for two factors: treatment (progesterone or vehicle) and group (sex/estrous cycle). When appropriate, ANOVA was followed by the Student–Newman–Keuls *post hoc* test. All results were expressed as mean±standard error of the mean (SEM). In all tests, the level of statistical significance was $P<0.05$.

3. Results

Acute or chronic progesterone treatment did not affect locomotor activity of rats, which ruled out any unspecific motor effects of progesterone in the doses used.

The antidepressant effects of the acute administration of progesterone in female and male rats during the FST were evaluated by a dose–response curve. The duration of immobility in the FST was not affected by any acute doses of progesterone (Fig. 1). The animals did not show any differences in time to perform movements in the pool, such as climbing and swimming, nor in immobile postures.

No abnormal changes in the estrous cycle were observed at any time during the chronic treatments.

Statistical analysis revealed a significant interaction between gender and treatment in immobility behaviors ($F_{(1,109)}=11.808$, $P<0.001$). In fact, chronic progesterone treatment decreased the

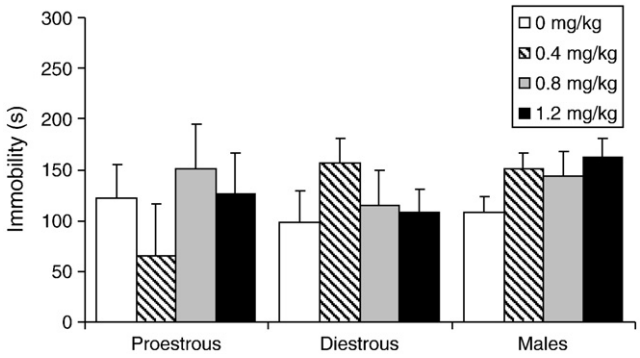

Fig. 1. Effects of acute progesterone administration on immobility behavior of rats submitted to the FST. $n=7$–9. Mean values±SEM for each group are shown. The duration of immobility was not altered by any dose of progesterone.