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Research report

Serotonergic receptor mechanisms underlying antidepressant-like action in the progesterone withdrawal model of hormonally induced depression in rats

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HIGHLIGHTS

- Progesterone withdrawal induces depression-like behavior in rat forced swim test.
- Different classes of antidepressants differently affect depression-like behavior.
- Depression-like behavior was sensitive to acute 5-HT_{1A}, 5-HT_3 and 5-HT_7 modulation.
- Receptor modulation effects were not additive to that of SERT inhibition.

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ABSTRACT

Hormonally induced mood disorders such as premenstrual dysphoric disorder (PMDD) are characterized by a range of physical and affective symptoms including anxiety, irritability, anhedonia, social withdrawal and depression. Studies demonstrated rodent models of progesterone withdrawal (PWD) have a high level of constructive and descriptive validity to model hormonally-induced mood disorders in women. Here we evaluate the effects of several classes of antidepressants in PWD female Long–Evans rats using the forced swim test (FST) as a measure of antidepressant activity. The study included fluoxetine, duloxetine, amitriptyline and an investigational multimodal antidepressant, vortioxetine (5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist; 5-HT_{1B} receptor partial agonist; 5-HT_{1A} receptor agonist; inhibitor of the serotonin transporter (SERT)). After 14 days of administration, amitriptyline and vortioxetine significantly reduced immobility in the FST whereas fluoxetine and duloxetine were ineffective. After 3 injections over 48 h, neither fluoxetine nor duloxetine reduced immobility, whereas amitriptyline and vortioxetine significantly reduced FST immobility during PWD. When administered acutely during PWD, the 5-HT_{1A} receptor agonist, flesinoxan, significantly reduced immobility, whereas the 5-HT_{1A} receptor antagonist, WAY-100635, increased immobility. The 5-HT_3 receptor antagonist, ondansetron, significantly reduced immobility, whereas the 5-HT_3 receptor agonist, SR-57227, increased immobility. The 5-HT_7 receptor antagonist, SB-269970, was inactive, although the 5-HT_7 receptor agonist, AS-19, significantly increased PWD-induced immobility. None of the compounds investigated (ondansetron, flesinoxan and SB-269970) improved the effect of fluoxetine during PWD. These data indicate that modulation of specific 5-HT receptor subtypes is critical for manipulating FST immobility in this model of hormone-induced depression.

1. Introduction

Hormonally induced mood disorders such as premenstrual dysphoric disorder (PMDD) are characterized by a range of physical and affective symptoms including anxiety, irritability, anhedonia, social withdrawal and depression. Cyclical premenstrual symptoms begin after ovulation (luteal phase) and resolve shortly after menstruation. Though negative mood, and specifically depression, is clearly correlated with long-term exposure to and rapid withdrawal from ovarian steroids, there are no robust or reproducible differences in the absolute amount of estrogens or progestins in symptomatic

Abbreviations: PMDD, premenstrual dysphoric disorder; PWD, progesterone withdrawal; FST, forced swim test; 5-HT, serotonin; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; SERT, serotonin transporter; AMI, amitriptyline; VOR, vortioxetine; FLX, fluoxetine; DLX, duloxetine; 8-OH-DPAT DPAT, 8-hydroxy-2-(di-N-propylamino)tetralin; FLES, flesinoxan; WAY, WAY-100635; OND, ondansetron; SR, SR-57227; SB, SB-269970; AS, AS-19; GABA, γ-aminobutyric acid; ANOVA, analysis of variance.

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vs. asymptomatic women [1]. It is, however, thought that long-term exposure to and withdrawal from progestins are sufficient to induce luteal phase depression [2–5] and these negative mood symptoms have been reliably modeled in rats using progesterone withdrawal (PWD) protocols and similar rodent models [6–9].

Exposure to exogenous progesterone followed by abrupt cessation in rodents is an established and well validated model of hormone-induced mood disorders. First, PWD results in a constellation of behavioral outcomes including depression-like behavior (increased immobility in forced swim test) [7,9–11], anhedonia (decreased preference for a palatable liquid) [8,9], altered social behavior [9,12], anxiety-like behavior (elevated plus maze, open field and acoustic startle test) [6,13–17] and irritability and aggression [18] that are analogous to the major diagnostic domains in humans. Second, in addition to this descriptive validity, the withdrawal model has also demonstrated construct validity. This includes the insensitivity to benzodiazepines exhibited during the luteal phase [19] which is also evident during PWD in rodents [15] and indicates altered GABA-A receptor expression. The model also has predictive validity as the increased startle response first described in rodents was later identified in humans [20,21].

Depressed mood, irrespective of etiology, is currently most often treated with selective serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs). Comparatively little is known about the role of serotonin (5-HT) in hormonally induced mood syndromes such as premenstrual syndrome, PMDD and post-partum depression or in rodent models of hormonally induced depression, which is a critical mechanistic link. Despite the widespread use of SSRIs and SNRIs for treatment of depression (including hormonally induced mood syndromes), meta-analyses of clinical studies indicate that there is a high rate of non-responders. Approximately 40% of patients do not have a positive response to SSRIs, and the response rate is typically not higher than 15–20% above placebo rates irrespective of whether the analysis was performed in a general major depressive disorder patient population or a population of PMDD patients [22–25]. Thus, studies directly comparing different classes and time courses of antidepressants in rodent models of hormonally induced depression would be informative.

The overall goal of the current study was to investigate the role of the 5-HT system in modifying depression-like behavior in the PWD model of hormonally induced depression. Our previous studies demonstrate robust and reproducible effects of progesterone withdrawal in the forced swim test [9], thus we utilized this assay to evaluate the efficacies of different classes of antidepressants after both chronic and acute administration, including an SSRI (fluoxetine), an SNRI (duloxetine), a tricyclic antidepressant (amitriptyline) and a novel investigational antidepressant (vortioxetine) in female rodents. Vortioxetine is a multimodal acting antidepressant modulating 5-HT receptor subtypes and inhibiting the 5-HT transporter (SERT). *In vitro* studies demonstrate that vortioxetine is a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist, and an inhibitor of the SERT [26,27]. To further explore the role of specific 5-HT receptors in depression-like behavior during PWD, we also investigated the effects of selective 5-HT_{1A}, 5-HT_3 and 5-HT_7 receptor ligands and combinations of fluoxetine with a selective 5-HT receptor modulator (5-HT_{1A} receptor agonist flesinoxan, 5-HT_3 receptor antagonist ondansetron, or 5-HT_7 receptor antagonist SB-269970).

2. Materials and methods

2.1. Animals

Female Long–Evans rats (125–150 g) were acquired from Charles River (Wilmington, MA) and group housed (3 per cage) under 12:12 light:dark cycle (lights on 6 am, lights off 6 pm). All female rats were gonadally intact as chronic administration of exogenous hormones in these doses abolishes normal estrous cyclicity and results in stable and predictable hormone levels [9].

Rats had *ad libitum* access to water and food. All animal experiments were conducted in accordance to the Institutional Animal Care and Use Committee of Lundbeck Research USA, following guidelines of NIH and AAALAC.

2.2. Forced swim test

The results of previous our studies demonstrated robust and reproducible effects of progesterone withdrawal in the forced swim test [9], thus we focused on this task in the current studies and depression-like behavior was assessed as previously described [9]. Briefly, female rats were brought into the testing room at least 1 h prior to testing. The forced swim test was conducted between 10 am and 5 pm, by immersing the rats in plexiglas cylinders (20 cm in diameter) filled with 23 °C water (30 cm deep) for 13 min. Immobility was manually scored using stopwatches for four 3-min time bins excluding the first minute of the trial. Immobility was defined as no movement other than those necessary to keep the animal's head above water. Results are presented either as amount of immobility in seconds for individual time bins (Fig. 2), or as the total immobility in seconds for the entire duration of the test (Figs. 3 and 4). In the double drug (fluoxetine + serotonin receptor modulator) experiments, data from the FST is illustrated as the percent of immobility of vehicle treated progesterone withdrawal group (% PWD immobility, Fig. 5).

2.3. Drug and hormone administration

Progesterone, amitriptyline, 8-OH-DPAT and other chemicals were obtained from Sigma–Aldrich (St. Louis, MO) unless otherwise stated. Sterile USP grade saline and water were obtained from APP Pharmaceuticals (Schaumburg, IL). Veterinary grade sesame oil was obtained from Wedgewood Pharmacy (Swedesboro, NJ). Fluoxetine, duloxetine, and vortioxetine were synthesized by H. Lundbeck A/S (Valby, Denmark). The 5-HT receptor modulators flesinoxan, WAY-100635, ondansetron, SR-57227, SB-269970, and AS-19 were purchased from Tocris Bioscience (Minneapolis, MN). All doses are expressed as mg/kg unless otherwise noted.

2.3.1. Progesterone withdrawal (PWD)

Only animals experiencing progesterone withdrawal were used in current study for several reasons. First, the data establishing the reliably increased depression-like behavior during PWD compared to oil injected control subjects have been previously published [9]. Typical data are illustrated in the supplemental figure (Fig. S1). Second, there is a floor effect in control subjects, such that level of baseline immobility in oil-injected control subjects is too low to effectively or sensitively detect any further decrease resulting from antidepressant administration [9]. Furthermore, when assessing the potential role of specific drugs to modulate depression-like behavior in hormonally induced depression, the relevant comparisons should be made in a model in which hormonally-induced depression is evident – i.e. PWD rats.

Progesterone was dissolved in sesame oil overnight at 37 °C with stirring. To induce progesterone withdrawal, all rats received 3 sets of i.p. injections of progesterone (6 mg/rat suspended in 0.2 ml sesame oil) on a 5 days on/2 days off schedule in a multiple withdrawal paradigm (schedule shown in Fig. 1) [9,28]. The forced swim test was conducted 48 h after final progesterone administration. Rats were only tested once in the forced swim test – the following studies were conducted in separate cohorts.