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Participation of the 5-HT_{1A} Receptor in the Antidepressant-Like Effect of Estrogens in the Forced Swimming Test

Erika Estrada-Camarena[1], Alonso Fernández-Guasti[2] and Carolina López-Rubalcava*[,2]

[1]Subdirección de Neurociencias, Instituto Nacional de Psiquiatría 'Ramón de la Fuente Muñíz', México City, DF, México; [2]Departamento de Farmacobiología, Centro de Investigación y de Estudios, Avanzados del IPN, México City, DF, Mexico

The aim of the present study was to explore the possible participation of the 5-HT_{1A} receptor in the antidepressant-like action of two estrogenic compounds: 17β-estradiol (E_2) and ethynil-estradiol (EE_2) in the FST. Ovariectomized female Wistar rats were used in all experiments. As a positive control, the effect of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n)-propil-aminotetraline (8-OH-DPAT; 0.0625, 0.125, 0.25 and 0.5 mg/kg) alone or in combination with WAY 100635 (0.5 and 1.0 mg/kg) was analyzed in the FST. In order to analyze the participation of the 5-HT_{1A} receptor in the antidepressant-like actions of estrogens, the effect of the selective antagonist WAY 100635 (0.5 and 1.0 mg/kg) in combination with E_2 (10 μg/rat) and EE_2 (5 μg/rat) was studied in the FST. In this case, WAY 100635 was administered either simultaneously with the estrogens (48 h before the FST test) or 30 min before the FST. On the other hand, a suboptimal dose of 8-OH-DPAT (0.0625 mg/kg), combined with a noneffective dose of E_2 (2.5 μg/rat) or EE_2 (1.25 μg/rat), was tested in the FST. The results showed that 8-OH-DPAT (0.25 and 0.5 mg/kg), E_2 (10 μg/rat), and EE_2 (5 μg/rat), by themselves, exerted an antidepressant-like action. The antagonist to the 5-HT_{1A} receptor WAY 100635, when applied together with 8-OH-DPAT or E_2, blocked their antidepressant-like actions, but not the one induced by EE_2. Interestingly, when the antagonist was applied 30 min before the FST, it was able to cancel the actions of EE_2 on immobility behavior, and had no effect on the actions of E_2. Finally, when a subthreshold dose of 8-OH-DPAT was combined with a noneffective dose of either E_2 or EE_2, an antidepressant-like action was observed. The results support the notion that the 5-HT_{1A} receptor is one of the mediators of the antidepressant-like action of E_2, and could indirectly contribute to the one induced by EE_2.
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INTRODUCTION

Specific receptor subtypes of the serotonergic and noradrenergic systems have been found to be involved in the antidepressant-like action of several antidepressant drugs. For example, it is accepted that the 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors all participate in the antidepressant-like action induced by selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine (FLX), paroxetine or sertraline (Blier, 2001; Blier and Ward, 2003; Duman *et al*, 1997; Li *et al*, 1996, 1997a,b; Rupprecht *et al*, 2001; Stahl, 1998a). In particular, the 5-HT_{1A} receptor has been proposed as a target of antidepressants that exhibit a clear therapeutic effect (for reviews see Blier and Ward, 2003; Cryan and Leonard, 2000). In this sense, *in vitro* and *in vivo* studies have shown that several SSRIs promote a desensitization of the presynaptic 5-HT_{1A} receptor subtype (Blier, 2001; Le Poul *et al*, 1995; Li *et al*, 1996, 1997a), and that facilitates a general enhancement of serotonin levels. These changes in serotonin transmission appear to promote either sensitization (Castro *et al*, 2003; Shen *et al*, 2002) or desensitization of the postsynaptic serotonergic 5-HT_{1A} receptors (Li *et al*, 1997a; Serres *et al*, 2000; Shen *et al*, 2002), depending on the brain area.

More evidence that sustains the participation of 5-HT_{1A} receptors in the effect of serotonergic antidepressants results from the use of antagonists to the 5-HT_{1A} receptor subtype, such as pindolol (Artigas *et al*, 1994, 1996) and WAY 100635 (Davidson and Stamford, 1995; Dawson and Nguyen, 1998; Gundlah *et al*, 1997; Hjorth *et al*, 1997; Millan *et al*, 1998), which, by blocking presynaptic serotonergic receptors, can facilitate the antidepressant action of serotonergic compounds in both clinical and preclinical studies (for a review, see Cryan and Leonard, 2000).

*Correspondence: Dr C López-Rubalcava, Departamento de Farmacobiología, Centro de Investigación y de Estudios, Avanzados IPN, Mexico City, DF 14330, Mexico, Tel: +52 55 5061 2872, Fax: +52 55 5061 2863, E-mail: clopezr@mail.cinvestav.mx
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Furthermore, several behavioral reports indicate that full or partial 5-HT$_{1A}$ agonists, such as buspirone, gepirone, indorenate, and 8-OH-DPAT, produce antidepressant-like effects in several animal models of depression, such as the FST and learned helplessness test (Lucki and Wieland, 1990; Lucki *et al*, 1994; Martin *et al*, 1990; Martínez-Mota *et al*, 2002; Wieland and Lucki, 1990).

The FST is an animal model used for the screening of antidepressant treatments, which was initially proposed as an animal model of depression (Lucki, 1997; Porsolt *et al*, 1977, 1978; Porsolt and Lenegre, 1992). Recently, it was proposed that, with the scoring of active behaviors in this model (for example, swimming and climbing) in addition to immobility behavior (indicative of the behavioral despair), it is possible to infer the neurotransmitter systems that mediate the antidepressant-like action of drugs (Detke *et al*, 1995; Lucki, 1997). Accordingly, noradrenergic re-uptake inhibitors like desipramine or maprotiline decrease the immobility behavior and increase at the same time climbing (Detke *et al*, 1995; Espejo and Miñano, 1999). In contrast, drugs that inhibit the re-uptake of serotonin, like FLX, sertraline, or paroxetine (Detke *et al*, 1995), agonists to the 5-HT$_{1A}$ receptor subtype, such as indorenate or 8-OH-DPAT (Martínez-Mota *et al*, 2002) and agonists to the 5-HT$_{2C}$ receptor subtype, such as WAY 161503, RO 60-0175, and RO 60-0332 (Cryan and Lucki, 2000), all decrease immobility and increase swimming behavior. In addition, the depletion of serotonin with para-chlorophenylalanine in rats cancels the actions of FLX, but not those of DMI (Page *et al*, 1999). On these bases, it appears that the FST is useful to identify the neurotransmitter system that mediates the antidepressant-like effect of a given drug.

Using this model (FST), the estrogens 17β-estradiol (E_2) and ethynil-estradiol (EE_2) have been found to produce antidepressant-like actions (Estrada-Camarena *et al*, 2003). An important difference between these two estrogens is that the natural estrogen E_2 is more potent at the serotonergic than at the noradrenergic system in decreasing re-uptake sites (Ghraf *et al*, 1983; Michel *et al*, 1987), while the synthetic steroidal compound EE_2 inhibits with a comparable efficacy the serotonergic and catecholaminergic sites (Ghraf *et al*, 1983; Michel *et al*, 1987). Both estrogens have been used in clinical practice as contraceptive and/or hormonal restitution therapies (Soares *et al*, 2001). In the FST, Estrada-Camarena *et al* (2003) reported that the behavioral profile induced by both estrogens was similar to that induced by the antidepressants FLX and desipramine. Thus, both E_2 and EE_2 decreased immobility behavior but, while E_2 promoted a concomitant increase in swimming, EE_2 increased both swimming and climbing behaviors.

Hence, considering the possible participation of the serotonergic system in the antidepressant-like action induced by these estrogens in the FST, the aim of the present work was to analyze the participation of the 5-HT$_{1A}$ receptor subtype in the antidepressant-like actions induced by E_2 and EE_2. In addition, it was evaluated whether the antidepressant action of estrogens on 5-HT$_{1A}$ receptors occurs immediately or requires several hours to be established. This receptor subtype was chosen, since *in vitro* and *in vivo* studies have shown that estrogens like E_2 and estradiol benzoate interact with pre- and postsynaptic 5-HT$_{1A}$ receptors. For example, estradiol can modulate the activity of this receptor depending on the dose used. In this sense, Jackson and Uphouse (1998) showed that the systemic administration of low doses of E_2 may synergize with low doses of 8-OH-DPAT to inhibit lordosis behavior (Jackson and Uphouse, 1996, 1998). However, when high doses of E_2 were administered, this compound blocked the actions of 8-OH-DPAT on lordosis behavior (Jackson and Uphouse, 1998; Uphouse, 2000). In relation to 5-HT$_{1A}$ presynaptic receptors, it has been shown that estradiol benzoate reduces the ability of 8-OH-DPAT to decrease the firing of dorsal raphe neurons (DRN) in rats (Lakoski, 1988) and promotes the desensitization of these receptors in the DRN of female monkeys (Lu and Bethea, 2002). As to the postsynaptic receptors, the effect of estrogens varies depending on the area studied and the treatment used. Thus, E_2 promotes a downregulation of mRNA expression and decreases the binding to this receptor subtype in some limbic areas such as the medial amygdala, the piriform cortex, and the perirhinal cortex (Österlund and Hurd, 1998), but not in others, such as the hippocampus and prefrontal cortex (Landry and Di Paolo, 2003). Interestingly, some of these actions have been reported to occur in minutes (Mize and Alper, 2000, 2001), while others appear after several hours (Raap *et al*, 2000; Uphouse, 2000). Finally, it is important to mention that, at present, there are no reports on the interaction between EE_2 and the 5-HT$_{1A}$ receptor subtype.

MATERIALS AND METHODS

Animals

Ovariectomized female Wistar rats (250–300 g, 3-months old), bred in the animal facilities of the 'Centro de Investigación y Estudios Avanzados' (Mexico City, Mexico), were housed in groups of six in polycarbonate cages and maintained on a 12 h light–dark cycle (lights on at 2200 h) in a temperature-controlled (22°C) room. Rats had free access to food and water, and were handled for 3–5 days prior to behavioral testing. All experimental procedures were performed in accordance with the Mexican official norm for animal care and handling (NOM-062-ZOO-1999) and approved by the Institutional Ethics Committee of the CINVESTAV-IPN.

Surgical Procedures

Ovariectomy was performed under ethylic ether anesthesia. Briefly, a single midline incision was made in the ventral area, oviducts were exposed, and ovaries removed. The complete extraction of the ovaries was corroborated by visual inspection. After 3 weeks, animals were randomly assigned to an experimental group and behavioral studies performed.

Forced Swimming Test

Swimming sessions were conducted by placing rats in individual glass cylinders (46 cm height $\times$ 20 cm diameter) containing water at 23–25°C, 30 cm deep, so that rats could not support themselves by touching the bottom with their

paws or tail. Two swimming sessions were conducted: an initial 15-min pre-test, followed 24 h later by a 5-min test. Following each swimming session, the rats were removed from the cylinders, dried with paper towels and placed into heated cages for 30 min, and then returned to their home cages. Test sessions were run between 1200 and 1500 h and videotaped for later scoring. A single observer, who was blind to the treatment conditions, did all the behavioral scoring.

Behavioral Scoring

A time-sampling technique was employed to score three different behaviors (Detke *et al*, 1995). During the test session, the scorer rated at the end of each 5-s period the following behaviors of the rat: (1) immobility—floating in the water without struggling, and doing only those movements necessary to keep the head above the water; (2) swimming—showing active swimming motions, more than those necessary to merely keep the head above water, that is, moving around in the cylinder or diving; and (3) climbing—presenting active movements with the forepaws in and out of the water, usually directed against the walls.

Drugs

The estrogenic compounds E_2 and EE_2 (Sigma-Aldrich, St Louis, MO, USA) were dissolved in corn oil and s.c. injected (0.2 ml/rat). (±)8-hydroxy-2-(di-n)-propil-aminotetraline hydrobromide (Sigma-Aldrich, St Louis, MO, USA) was dissolved in saline solution, whereas WAY 100635 maleate (Sigma-Aldrich, St Louis, MO, USA) was dissolved in distilled water and s.c. (2 ml/kg) administered. All solutions were freshly prepared before each experimental series.

Experimental Design

Experiment 1. Participation of the 5-HT$_{1A}$ receptor in the antidepressant-like action of 8-OH-DPAT. In the first experiment, the effect of an agonist and a selective antagonist to the 5-HT$_{1A}$ receptor subtype, 8-OH-DPAT, and WAY 100635, respectively, were evaluated in ovariectomized female rats subjected to the FST. It is important to mention that, in previous studies, it has been reported that it is necessary to use a subacute schedule of high doses of antidepressants in order to observe the antidepressant-like actions in the FST (Detke *et al*, 1997). Therefore, independent groups ($n = 7$–13) were formed and received three injections between the FST pretest and test sessions of one of the following doses of 8-OH-DPAT: 0.0625, 0.125, 0.25, and 0.5 mg/kg, or WAY 100635 (0.5 and 1.0 mg/kg) at 23, 5, and 1 h before the test session.

In a second experiment, the effect of WAY 100635 on the action of 8-OH-DPAT was analyzed ($n = 7$–12). In this case, two doses of WAY 100635 (0.5 and 1.0 mg/kg) were combined with an effective dose of 8-OH-DPAT (0.5 mg/kg). Both compounds were administered simultaneously at 23, 5, and 1 h before the FST.

Control groups were treated with distilled water and saline solution following the same schedule of administration as the experimental groups.

Experiment 2. Effect of WAY 100635 on the antidepressant-like actions of E_2 and EE_2. Since estrogens have effects on serotonergic receptors after both short (in terms of minutes) and long (in terms of hours or few days) latencies, and in order to evaluate the possible involvement of the 5-HT$_{1A}$ receptor in the antidepressant-like effect of E_2 and EE_2 in the FST, the experiment was divided into two parts. In the first one, designed to study the short-term effects of estrogens on 5-HT$_{1A}$ receptors, WAY 100635 was administered simultaneously with an effective dose of E_2 (10 μg/rat, $n = 8$–10) or EE_2 (5 μg/rat, $n = 6$–10) and the test was performed 48 h later. Doses and latencies were chosen according to previous studies (Estrada-Camarena *et al*, 2003). In this experiment, the animals were assigned to independent groups that received one of the following treatments: saline + corn-oil (control), E_2 (10 μg/rat) + saline, E_2 (10 μg/rat) + WAY 100635 (0.5 and 1.0 mg/kg), EE_2 (5 μg/rat) + saline, EE_2 (5 μg/rat) + WAY 100635 (0.5 and 1.0 mg/kg). In the second part, aimed to study the long-term actions of estrogens on this serotonergic receptor subtype, E_2 or EE_2 ($n = 6$–10) was administered 48 h before the FST and WAY 100635 was injected 30 min before the test session. The experimental groups included in this experiment were saline + corn-oil (control), E_2 (10 μg/rat) + saline, E_2 (10 μg/rat) + WAY 100635 (1 mg/kg), EE_2 (5 μg/rat) + saline, EE_2 (5 μg/kg) + WAY 100635 (1 mg/kg) (see Figure 1).

Experiment 3. Effect of the combination of the 8-OH-DPAT with E_2 or EE_2. This experiment was designed to establish if the effect of a subthreshold dose of the 5-HT$_{1A}$ agonist 8-OH-DPAT could synergize with that of a noneffective dose of E_2 or EE_2 to produce an antidepressant-like action in the FST. As aforementioned, the actions produced by estrogens on the serotonergic system may involve minutes to days. On this basis, as in the previous experiment, the putative synergism of estrogens with 8-OH-DPAT was analyzed at two different times. In the first part, E_2 ($n = 7$–11) or EE_2 ($n = 8$–11) was applied 48 h before the FST and 8-OH-DPAT (0.0625 mg/kg) at 23, 5, and 1 h before the FST. In this case, the experimental groups were: saline + corn-oil (control), 8-OH-DPAT (0.0625 mg/kg) + corn-oil, E_2 (2.5 μg/rat) + saline, E_2 (2.5 μg/rat) + 8-OH-DPAT (0.0625 mg/kg), EE_2 (1.25 μg/rat) + saline, and EE_2 (1.25 μg/rat) + 8-OH-DPAT (0.0625 mg/kg). In the second part, only one injection of 8-OH-DPAT and one of estrogen were applied simultaneously 48 h before the FST.

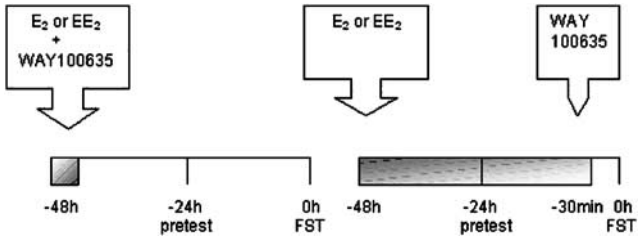

Figure 1 Schedules of antagonist administrations in experiment 2. In order to evaluate the short-term actions of estrogens, steroids and the 5-HT$_{1A}$ receptor antagonist WAY 100635 were administered simultaneously (left panel). For the evaluation of long-term actions of estrogens, steroids were administered 47.5 h before the antagonist (right panel).

For this experiment, the groups used were: saline + corn oil (control), corn oil + 8-OH-DPAT (0.0625 mg/kg), E_2 (2.5 μg/rat) + 8-OH-DPAT (0.0625 mg/kg), EE_2 (1.25 μg/rat) + 8-OH-DPAT (0.0625 mg/kg).

Doses and latencies for 8-OH-DPAT, WAY 100635, and estrogens were selected on the basis of previous reports (Estrada-Camarena *et al*, 2003; Martínez-Mota *et al*, 2002) and the present study.

Statistics

Statistical analyses were performed using the SigmaStat 2.03. computational program. A one-way ANOVA on ranks, Kruskall–Wallis test followed by the Dunn's test, was used, and only the values with $p<0.05$ were accepted as significant.

RESULTS

Figure 2 shows the effect of several doses of 8-OH-DPAT (panel a), WAY 100635 (panel b), and the combination of both drugs (panel c) in the FST. As it can be seen, 8-OH-DPAT significantly decreased the immobility behavior ($H=16.90$, df = 4, $p=0.002$) and increased the swimming behavior ($H=19.92$, df = 4, $p<0.001$). This effect reached statistical significance at doses of 0.25 and 0.5 mg/kg. No changes were observed in the climbing behavior after 8-OH-DPAT ($H=3.91$, df = 4, NS). On the other hand, the antagonist to the 5-HT$_{1A}$ receptor subtype, WAY 100635, significantly decreased swimming behavior at the dose of 1.0 mg/kg in the FST (one-way ANOVA values for immobility, $H=4.69$, df = 2, NS; for swimming, $H=15.11$, df = 2, $p<0.001$; and for climbing, $H=0.059$, df = 2, NS). Finally, in panel c, the effect of WAY 100635 on the antidepressant-like action induced by an effective dose of 8-OH-DPAT can be observed. The decrease of immobility behavior and the concomitant increase of swimming behavior induced by 8-OH-DPAT were completely blocked by WAY 100635 at the two doses used. Significant changes in the climbing behavior were not observed (one-way ANOVA values for immobility, $H=14.98$, df = 3, $p=0.002$; swimming, $H=24.3$, df = 3, $p<0.001$; and climbing, $H=5.73$, df = 3, NS).

Figure 3 shows the effect of the simultaneous application, 48 h before the FST, of the selective antagonist to the 5-HT$_{1A}$ receptor, WAY 100635, and E_2 (panel a) or EE_2 (panel b). As it can be seen, the decrease in immobility behavior and the increase in swimming behavior induced by an effective dose of E_2 (10 μg/rat, panel a) were blocked by both doses of WAY 100635 (0.5 and 1.0 mg/kg). There were no changes in the climbing behavior (one-way ANOVA for the immobility behavior, $H=17.3$, df = 3, $p<0.001$, for swimming behavior, $H=17.59$, df = 3, $p<0.001$, and for climbing, $H=0.85$, df = 3, NS). In relation to the effect of WAY 100635 on the actions of EE_2 (panel b), the results showed that WAY 100635 was unable to cancel the effect of this steroid on immobility, swimming, and climbing behaviors (one-way ANOVA for immobility, $H=14.44$, df = 3, $p=0.002$; for swimming, $H=11.26$, df = 3, $p=0.01$; for climbing, $H=4.87$, df = 3, NS).

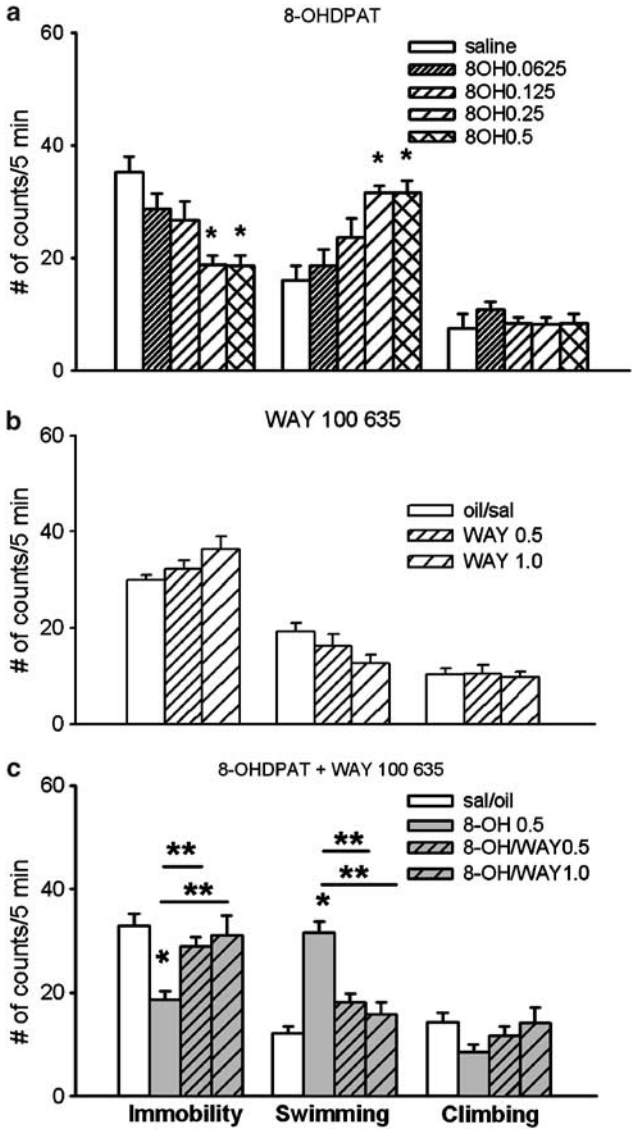

Figure 2 Behavioral effects produced by several doses of 8-OH-DPAT (8-OH, −23, −5, and −1 h; panel a), WAY 100635 (WAY, −23, −5, and −1 h, panel b) and 8-OH plus WAY 100635 (−23, −5, and −1 h, panel c) in the FST. Data correspond to mean of counts (+1SEM) of immobility, swimming, and climbing behaviors when sampled every 5 s during a 5-min test period (7–13 animals per group). Dunn's test, *$p<0.05$.

The effects of the application of WAY 100635 several hours (47.5 h) after injecting E_2 or EE_2 are shown in Table 1. As it can be seen, WAY 100635 did not cancel the antidepressant-like action of the natural estrogen, E_2. Thus, the decrease in the immobility behavior accompanied by the increase in swimming behavior remained in the group that received WAY 100635 several hours after E_2. In contrast, under this administration schedule, the antagonist to the 5-HT$_{1A}$ receptor subtype blocked the effects of EE_2. In this case, when WAY 100635 was applied several hours after EE_2, the steroid did not decrease the immobility behavior in comparison to the control group. In addition, a decrease in swimming behavior was observed. One-way ANOVA on ranks is shown in Table 1.

Figure 4 illustrates the effect of the application of estrogens, E_2 (panel a) and EE_2 (panel b), 48 h before the

FST, followed by the administration of three injections of a suboptimal dose of 8-OH-DPAT. Low doses of either E_2 or 8-OH-DPAT had no effects in the FST (panel a); however, when combined, a decrease in immobility behavior ($H = 15.74$, df = 3, $p = 0.001$) with a concomitant increase in swimming behavior ($H = 15.54$, df = 3, $p = 0.001$) and no changes in climbing behavior ($H = 1.99$, df = 3, NS) were observed. A similar effect was found when a suboptimal dose of the synthetic estrogen EE_2 was combined with a noneffective treatment of 8-OH-DPAT (panel b), that is,

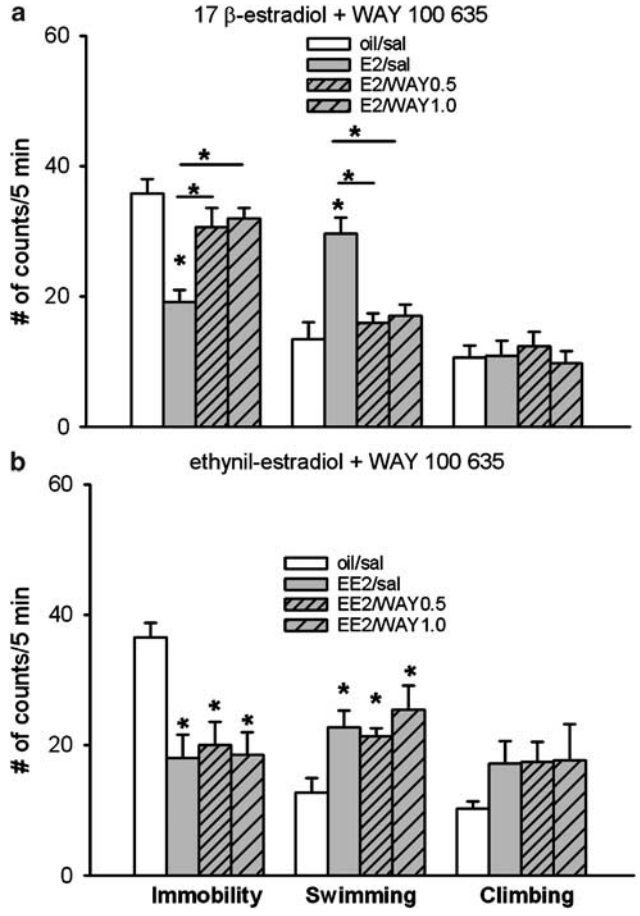

Figure 3 Behavioral effect of the simultaneous application of WAY 100635 (WAY; −48 h) and an effective dose of E_2 (−48 h, panel a) or EE_2 (−48 h; panel b) in the FST. Data represent the mean of counts (+1SEM) of immobility, swimming, and climbing behaviors when sampled every 5 s during a 5-min test period (6–10 animals per group). Dunn's test, $^*p < 0.05$.

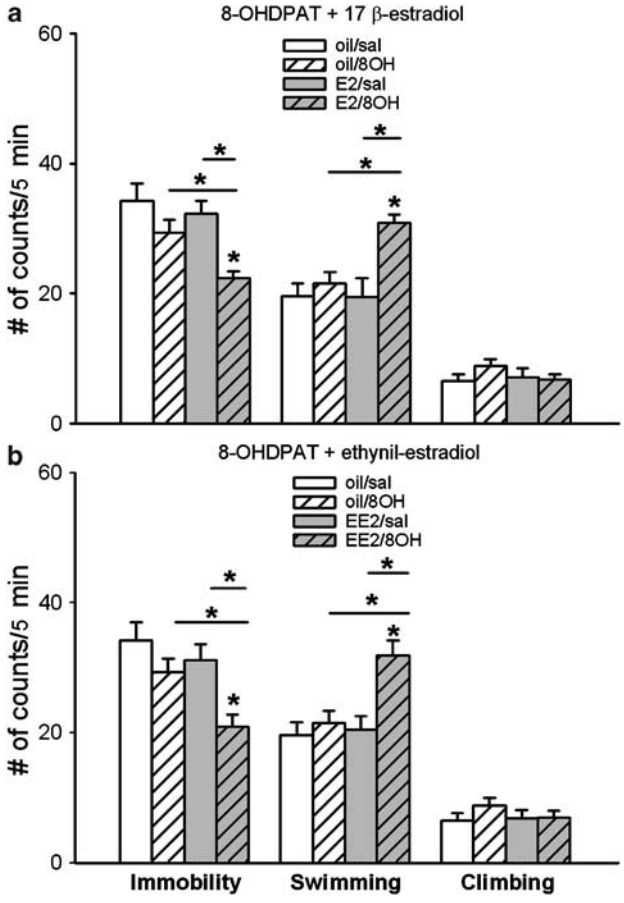

Figure 4 Behavioral effect of the combination of a subthreshold dose of E_2 (−48 h; panel a) or EE_2 (−48 h, panel b) with noneffective doses of 8-OH-DPAT (8-OH; −23, −5, and −1 h) in the FST. Data represent the mean of counts (+1SEM) of immobility, swimming, and climbing behaviors when sampled every 5 s during a 5-min test period (7–11 animals per group). Dunn's test, $^*p < 0.05$.

Table 1 Effect of the WAY 100635 Applied 30 min before the FST on the Antidepressant-Like Action of E_2 or EE_2

Treatments	Immobility	Swimming	Climbing	*n*
Oil/saline	31.90±1.14	19.50±1.20	9.30±1.06	10
E_2/saline	20.18±2.20*	28.18±2.58*	10.72±2.12	11
E_2/WAY 100 635	24.41±1.14*	24.5±1.8	10.08±1.79	12
One-way ANOVA on ranks	$H = 14.24$, df = 2, $p < 0.001$	$H = 6.89$, df = 2, $p = 0.03$	$H = 0.03$, df = 2 NS	
EE_2/saline	21.16±2.56*	21.19±1.70	15.50±2.25	12
EE_2/WAY 100 635	29.90±2.90	14.09±1.76**	14.45±2.28	11
One-way ANOVA on ranks	$H = 8.67$, df = 2, $p = 0.01$	$H = 8.74$, df = 2, $p = 0.005$	$H = 5.66$, df = 2, NS	

The table shows the mean±SEM of immobility, swimming, and climbing when sampled every 5 s during 5 min of FST session. WAY 100635 (1 mg/kg) was applied 47.5 h after E_2 (10 μg/rat) or EE_2 (5 μg/rat). Steroids were administered 48 h before the FST. Dunn's test.
$^*p < 0.05$ *vs* control group (oil/saline).
$^{**}p < 0.05$ *vs* EE_2/saline.

Table 2 Effect of the Simultaneous Administration of 8-OH-DPAT and E_2 or EE_2 48 h before the FST

Treatments	Immobility	Swimming	Climbing	*n*
Oil/saline	38.2 ± 1.6	9.6 ± 1.7	14.4 ± 2.3	9
Oil/8-OH-DPAT	39.6 ± 2.1	5.3 ± 1.0	16.2 ± 3.2	9
E2/8-OH-DPAT	29.5 ± 2.9***	10.5 ± 2.3	17.7 ± 2.0	10
EE2/8-OH-DPAT	28.9 ± 2.2***	14.8 ± 2.8**	15.3 ± 2.0	10
One-way ANOVA	$H = 13.23$, df = 3, $p = 0.004$	$H = 8.49$, df = 3, $p = 0.03$	$H = 1.38$, df = 3, NS	

The table shows mean ± SEM of immobility, swimming and climbing when sampled every 5 s during 5 min of FST session of the combination of 8-OH-DPAT (0.0625 mg/kg, s.c.) with E_2 (2.5 μg/rat, s.c.) or EE_2 (1.25 μg/rat, s.c.). Both, estrogens and 8-OH-DPAT, were administered simultaneously 48 h before FST. Dunn's test.
*$p < 0.05$ *vs* oil/saline.
**$p < 0.05$ *vs* oil/8HDPAT.

a decrease in immobility behavior ($H = 13.23$, df = 3, $p = 0.004$) accompanied by an increase of swimming behavior ($H = 12.55$, df = 3, $p = 0.006$) was observed. None of the treatments induced changes in climbing behavior ($H = 2.02$, df = 3, NS).

The results obtained after the simultaneous injection of a suboptimal dose of 8-OH-DPAT with a noneffective dose of E_2 or EE_2 48 h before the FST can be seen in Table 2. Administration of the agonist to the 5-HT$_{1A}$ receptor subtype with estrogens elicited a clear antidepressant-like action ($p < 0.05$), and promoted a slight increase in both swimming and climbing behaviors, without reaching statistical significance. For one-way ANOVA test, see Table 2.

DISCUSSION

In the present study, the 5-HT$_{1A}$ agonist 8-OH-DPAT produced an antidepressant-like action in ovariectomized female rats in the FST, characterized by a decrease in immobility behavior and an increase in swimming behavior. A similar behavioral profile has been reported for male rats treated with the SSRIs, indorenate (a full agonist to the 5-HT$_{1A}$ receptor) and buspirone (a partial agonist to 5-HT$_{1A}$ receptors) (Detke *et al*, 1995; Martínez-Mota *et al*, 2002). However, female rats seem more sensitive than males to the actions of 8-OH-DPAT, since lower doses of this compound were needed in the present study to induce an antidepressant-like action in the FST. Hence, the behavioral profile found with 8-OH-DPAT by other groups in male rats can be extended to ovariectomized female rats. In the present study, the antidepressant-like action of 8-OH-DPAT was completely blocked by the selective antagonist to the 5-HT$_{1A}$ receptor, WAY 100635 (Forster *et al*, 1995), a result that confirms the involvement of this receptor subtype in the antidepressant-like effect of 8-OH-DPAT in female rats.

As mentioned before, the behavioral profile of drugs acting at the serotonergic system, including agonists to 5-HT$_{1A}$ receptors, is a decrease in inmmobility and a concomitant increase in swimming behavior in the FST (Detke *et al*, 1995; Cryan and Lucki, 2000; Martínez-Mota *et al*, 2002; Page *et al*, 1999). In relation to the effects of estrogens, in the present study, it was observed that the antidepressant-like action of E_2 was characterized by an increase in swimming, whereas that of EE_2 was distinguished by an increase in both swimming and climbing behaviors. Hence, it is reasonable to consider that the serotonergic system contributes to the antidepressant-like actions of both estrogenic compounds. In the present series of experiments, it was also found that the administration of suboptimal doses of 8-OH-DPAT combined with noneffective doses of E_2 or EE_2 resulted in clear antidepressant-like effects. These results suggest that both compounds, steroids and the 5-HT$_{1A}$ receptor agonist, could act together to promote the antidepressant-like action observed. Jackson and Uphouse (1998) reported a similar result when showing that low doses of E_2 combined with low doses of 8-OH-DPAT synergized to inhibit lordosis behavior. Notwithstanding, it is possible that the interaction between estrogens and the 5-HT$_{1A}$ receptor subtype may be different for each type of estrogen (*vide infra*).

Present results showed that E_2: (1) produced a behavioral profile similar to that induced by an agonist to the 5-HT$_{1A}$ receptor, 8-OH-DPAT, in the FST; (2) synergized with 8-OH-DPAT to induce an antidepressant-like action and, finally, estradiol's antidepressant-like effect was totally cancelled by the simultaneous administration of the selective 5-HT$_{1A}$ antagonist, WAY 100635. Therefore, it can be concluded that the 5-HT$_{1A}$ receptor subtype is a mediator of the antidepressant-like action of E_2. Evidences in favor of this hypothesis are the results derived from *in vitro* and *in vivo* studies, showing that the 5-HT$_{1A}$ receptor subtype is a target of estrogens and some estrogen-like compounds (Bethea *et al*, 1998; Biegon *et al*, 1983; Mize and Alper, 2000; Mize *et al*, 2001; Raap *et al*, 2000; Uphouse, 2000). Indeed, Lu and Bethea (2002) demonstrated that E_2 induces the desensitization of 5-HT$_{1A}$ receptors in the DRN of monkeys. Thus, it is possible that the antidepressant-like actions of E_2 are mediated by its action on these presynaptic receptors. Interestingly, it has also been reported that E_2 may induce changes at postsynaptic 5-HT$_{1A}$ receptors. In this sense, in ovariectomized Flinders rats (a genetic model of depression), the characteristic increased mRNA expression of 5-HT$_{1A}$ receptors in some limbic areas such as amygdala, hypothalamus, and hippocampus, is reduced by a single injection of E_2 (Österlund *et al*, 1999). Furthermore, in Wistar female rats, ovariectomy induces an increase in the sensitivity of 5-HT$_{1A}$ receptors in the hypothalamus that is abolished by E_2 treatment (Raap *et al*, 2002). Taken together, these results support the notion that the actions of E_2 on 5-HT$_{1A}$ receptors may promote adaptive changes

similar to those induced by antidepressants to exert their effects. Interestingly, the occurrence of these adaptive changes may take less time for estrogens than for antidepressants (Raap *et al*, 2000).

At least two mechanisms have been proposed to explain the actions of estrogens. The first one involves the activation of intracellular steroid receptors, while the second one implies the activation of membrane receptors, with the subsequent activation of second messenger systems (for a review, see Lee and McEwen, 2001). Considering this, it could be expected that E_2 stimulates the 5-HT$_{1A}$ receptor subtype in two different manners: one mechanism, underlying the effects that last from several hours to days, that could involve the activation of intracellular estrogen receptors and the subsequent transcription of the gene that codifies for the 5-HT$_{1A}$ receptor subtype. The other mechanism, underlying those estrogenic effects that appear in the order of minutes, that implies the activation of the 5-HT$_{1A}$ receptor subtype and its respective second messenger cascade (Mize and Alper, 2000). The fact that the antidepressant-like actions of E_2 were blocked by WAY 100635 when both compounds were administered simultaneously (48 h before the test) indicates that the 5-HT$_{1A}$ receptor subtype has a significant participation in the anti-immobility effect induced by this steroid, suggesting a direct interaction with 5-HT$_{1A}$ receptors. This hypothesis could find support in the fact that the application of WAY 100635, 30 min before the test (or 47.5 h after E_2), did not block the antidepressant-like action of the steroid. Taken together, these results suggest that under these circumstances E_2 may compete with the antagonist for the occupancy of the 5-HT$_{1A}$ receptor, and in this case the action of E_2 would not be intracellularly mediated. Another possibility is that E_2 activates other type of receptors, indirectly promoting the activation of the 5-HT$_{1A}$ receptor subtype like it has been suggested by *in vitro* studies (Mize and Alper, 2002). In this case, the process could require several minutes before the 5-HT$_{1A}$ receptor is activated by a heterologous process, a time sufficient for WAY 100635 to occupy 5-HT$_{1A}$ receptors and cancel its activation. Notwithstanding, specific experiments are necessary to explore both possibilities, the present results are in favor of a direct interaction between E_2 and 5-HT$_{1A}$ receptors, that is not mediated by the intracellular estrogen receptor.

In relation to the antidepressant-like actions of EE_2, the participation of the 5-HT$_{1A}$ receptor seems to be different from that of E_2. In this case, it was found that: (1) the behavioral profile of EE_2 was different from that induced by 8-OH-DPAT and E_2 in the FST; (2) the combination of subthreshold doses of both 8-OH-DPAT and EE_2 promoted an antidepressant-like action in the FST; and (3) WAY 100635 was unable to cancel the antidepressant-like effects of EE_2 when simultaneously applied (48 h before the test), but blocked it when applied 47.5 h after the steroid (30 min before the test). The present results suggest the activation of the 5-HT$_{1A}$ receptor subtype. It is possible that the stimulation of this serotonergic receptor by EE_2 is secondary to the activation of other unknown sites. In this point, it is important to mention that the behavioral profile induced by EE_2 alone was similar to that produced by mixed reuptake inhibitors that act at both the noradrenergic and serotonergic systems, such as venlafaxine and duloxetine in the FST, that is, these compounds produce a decrease in immobility with a concomitant increase in both swimming and climbing behaviors (Rénéric and Lucki, 1998). Furthermore, *in vitro* studies have shown that EE_2 inhibits both serotonin and noradrenaline transporters with a similar potency (Michel *et al*, 1987; Ghraf *et al*, 1983). At present, data on the mechanism of action of EE_2 on neurotransmitter systems are derived only from *in vitro* studies and there are no data from behavioral studies. The reasons why the noradrenergic actions are not as evident as the serotonergic ones are unknown; however, it is important to mention that there is a marked tendency of EE_2 to increase climbing behavior when compared with the control group, a datum pointing towards the participation of the noradrenergic system. At present, no data in relation to this point exist; therefore, future studies should be undertaken to analyze the possible sites of action other than estrogenic receptors for EE_2.

The administration of a subthreshold dose of E_2 or EE_2 together with a subacute 8-OH-DPAT treatment (three injections between pre-test and test sessions) promotes an antidepressant-like action characterized by a decrease in immobility, accompanied by an increase in swimming behavior. Since E_2 promotes a behavioral profile similar to that induced by 8-OH-DPAT itself, it is possible to suggest that both compounds act at the 5-HT$_{1A}$ receptor subtype. In the case of the combination of EE_2 and 8-OH-DPAT, it is possible that the specific action of 8-OH-DPAT at the 5-HT$_{1A}$ receptor subtype, after a subacute treatment, masked the behavioral profile induced by EE_2, for example, an increase in swimming instead of an increase in both swimming and climbing behaviors. Moreover, when an injection of EE_2 was combined with one of 8-OH-DPAT (48 h before the test), the antidepressant-like action observed was characterized by a decrease in immobility behavior, accompanied by an increase in both swimming and climbing behaviors. Hence, it is possible to consider that, under this experimental schedule, the behavioral profile of EE_2 prevails over that induced by 8-OH-DPAT.

Changes in the sensitivity of the 5-HT$_{1A}$ receptor have been associated with the antidepressant action of several compounds in clinical and preclinical studies (for reviews, see Blier and Ward, 2003; Cryan and Leonard, 2000; Duman *et al*, 1997). Recently, it was shown that the application of a high dose of 8-OH-DPAT (0.5 mg/kg) or FLX (10 mg/kg) desensitized presynaptic 5-HT$_{1A}$ receptors in a short time (Kennett *et al*, 1987; Riad *et al*, 2001, 2004). These results suggest that the overstimulation of the 5-HT$_{1A}$ receptor could accelerate its desensitization and an antidepressant action would follow with a shorter latency. In the present study, it was observed that a low dose of 8-OH-DPAT by itself was insufficient to induce an antidepressant-like action; however, when this agonist was combined with a noneffective dose of E_2 or EE_2, administered 48 h before the FST, a clear antidepressant-like action was observed. Hence, it is possible that the combined treatment of compounds which act (directly or indirectly) on the 5-HT$_{1A}$ receptor subtype could synergize to promote changes in its sensitivity that result in antidepressant-like effects.

In conclusion, the 5-HT$_{1A}$ receptor seems to be a target of E_2 and EE_2 in their antidepressant-like actions. This receptor subtype has been considered to play a role in the

physiology of depression and in the mechanism of action of several types of antidepressants (Blier and Ward, 2003; Duman, 1999; Stahl, 1998a). Recently, some groups proposed that combining antidepressants with other compounds that promote the desensitization of 5-HT$_{1A}$ receptors could shorten the latency for the onset of SSRI's therapeutic effects (Artigas *et al*, 1994, 1996; Blier and de Montigny, 1994; Cryan and Leonard, 2000). From this perspective, the administration of E_2, an estrogenic compound that acts at the 5-HT$_{1A}$ receptor subtype, could be an alternative to improve the action of antidepressants. Indeed, some clinical studies have combined estrogens with FLX, sertraline or citalopram, with positive results (Stahl, 1998b; Schneider *et al*, 1997, 2001; Soares *et al*, 2001).

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