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Antidepressant-Like Effect of Different Estrogenic Compounds in the Forced Swimming Test

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The present study evaluated the possible antidepressant-like action of the natural estrogen 17β-estradiol (E_2, 2.5–10 μg/rat), the synthetic steroidal estrogen ethinyl-estradiol (EE_2, 1.25–10.0 μg/rat), and the nonsteroidal synthetic estrogen, diethyl-stilbestrol (DES, 0.25–1.0 mg/rat) in ovariectomized adult female Wistar rats using the forced swimming test (FST). The behavioral profile induced by the estrogens was compared with that induced by the antidepressants fluoxetine (FLX, 2.5–10 mg/kg) and desipramine (DMI, 2.5–10 mg/kg). In addition, the temporal course of the antidepressant-like action of the estrogenic compounds was analyzed. FLX and DMI induced an antidepressant-like effect characterized by a reduced immobility and increased swimming for FLX and decreased immobility and increased climbing for DMI. Both E_2 and EE_2 produced a decrease in immobility and an increase in swimming, suggesting an antidepressant-like action. DES did not affect the responses in this animal model of depression at any dose tested. The time course analysis of the actions of E_2 (10 μg/rat) and EE_2 (5 μg/rat) showed that both compounds induced an antidepressant-like effect observed 1 h after their injection lasting for 2–3 days.
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INTRODUCTION

Clinical evidence suggests that the vulnerability to depression of some women is associated with the hormonal fluctuations during their life, in which estrogens play an important role (Halbreich *et al*, 1986; Hamilton *et al*, 1988; Hendrick *et al*, 1998; Oppenheim, 1983; Stahl 1997, 1998b). For example, chronic treatment of these women with 17β-estradiol (E_2) or conjugated equine estrogens decreased depressive symptoms during the perimenopausal and postpartum periods (Ahonkas *et al*, 1999; Epperson *et al*, 1999; Genazzani *et al*, 1999; Klaiber *et al*, 1979; López-Jaramillo *et al*, 1996; Österlund and Hurd, 2001). Indeed, it has been suggested that estrogens could shorten or improve the therapeutic action of some antidepressants (Prange, 1972; Schneider *et al*, 1997, 2001). Furthermore, preclinical reports refer that E_2 produces antidepressant-like actions in animal models of depression such as the forced swimming and the tail suspension tests (Bernardi *et al*, 1989; Galea *et al*, 2001; Okada *et al*, 1997; Rachman *et al*, 1998).

Antidepressants such as the selective serotonin reuptake inhibitors (SSRIs; ie fluoxetine (FLX), sertraline) and catecholamine reuptake inhibitors (ie desipramine (DMI), maprotiline, bupropion) produce initially a blockade of their respective reuptake sites, thereby enhancing the neurotransmitter availability (Hervás and Artigas, 1998; Kreiss and Lucki, 1995; Linner *et al*, 1999; Piñeyro and Blier, 1999; Stahl, 1998a). Later, this increased availability promotes adaptive changes on specific receptor systems, as it has been shown for the 5-HT_{1A}, 5-$HT_{2A/C}$ and for the noradrenergic α_2 and β receptor subtypes (Blier, 2001; Blier and Montigny, 1994; Chaput *et al*, 1991; Dawson and Nguyen, 1998; Duman, 1999; Gobert and Millan, 1999; Le Poul *et al*, 2000; Stahl, 1998a). On the other hand, *in vitro* studies demonstrate that estrogens can interact, at several levels, with the dopaminergic, the noradrenergic, and the serotonergic systems (Attali *et al*, 1997; Bethea *et al*, 1998; Biegon *et al*, 1983; Cyr *et al*, 2000; McEwen, 1999; Rubinow *et al*, 1998). For example, estrogens can inhibit the function of the monoaminergic transporter (Ghraf *et al*, 1983; Michel *et al*, 1987; Wilson *et al*, 1988) and interact with either 5-HT_{1A}, 5-HT_{2A}, or β-adrenergic receptors in several brain areas (Biegon *et al*, 1983; Fink *et al*, 1996; Kendall *et al*, 1982; Mize and Alper, 2000; Österlund *et al*, 1999; Raap *et al*, 2000; Sumner and Fink, 1995). Interestingly, estrogenic compounds modify those neurotransmitter systems that are targets of antidepressant treatments.

It is important to mention that the estrogenic activity on each of these systems varies depending on the estrogen nature (Chang and Chang, 1999; Ghraf *et al*, 1983; Michel *et al*, 1987). Thus, the natural estrogen E_2 decreases both serotonin and noradrenaline reuptake sites, being more

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potent at the serotonergic than at the noradrenergic system (Ghraf *et al*, 1983; Michel *et al*, 1987). By contrast, the synthetic steroidal compound, ethynil-estradiol (EE_2), inhibits, with comparable efficacy, both the serotonergic and the catecholaminergic transporters (Ghraf *et al*, 1983; Michel *et al*, 1987). Finally, the nonsteroidal synthetic estrogen diethyl-stilbestrol (DES) decreases dopaminergic and noradrenergic reuptake sites (Ghraf *et al*, 1983) without producing changes in the number or affinity of serotonergic transporters (Chang and Chang, 1999).

On these bases, the main purpose of the present study was to investigate the possible antidepressant-like effect of three types of estrogens: a natural estrogen (E_2); a synthetic steroidal estrogen (EE_2); and a synthetic nonsteroidal estrogen (DES) in the forced swimming test (FST).

The FST is an animal model commonly used for both the screening of antidepressant drugs (Borsini, 1995; Borsini and Meli, 1988; Porsolt and Lenégre, 1992) and the analysis of the neurobiological bases of depression (Duncan *et al*, 1985; Paul *et al*, 1990). In this model, rats are forced to swim and eventually adopt a floating posture identified as immobility behavior (Borsini and Meli, 1988; Porsolt *et al*, 1977; Porsolt and Lenégre, 1992), which is considered as an index of 'behavioral despair' (Porsolt *et al*, 1977, 1978; Porsolt and Lenégre, 1992). In the FST, antidepressants induce a decrease in immobility (Borsini and Meli, 1988; Kitada *et al*, 1983; Porsolt *et al*, 1977). Detke *et al* (1995) pointed out that in addition to immobility, it is also possible to register two active behaviors, swimming and climbing, which appear to be sensitive to specific antidepressant drug classes (Detke *et al*, 1995; Lucki, 1997). Thus, SSRIs such as FLX, paroxetine, or sertraline decrease immobility and increase swimming (Cryan and Lucki, 2000; Detke *et al*, 1995; Espejo and Minano, 1999; Page *et al*, 1999), while the selective noradrenergic and dopaminergic reuptake inhibitors, like DMI, maprotiline, and bupropion, decrease immobility accompanied by an increase in climbing behavior (Detke *et al*, 1995; Espejo and Minano, 1999; Hemby *et al*, 1997; Rénéric and Lucki, 1998). Hence a second purpose of this investigation was to determine the behavioral profiles induced by the estrogenic compounds and compare them with those produced by the classic antidepressants FLX and DMI in the FST.

Finally, several authors suggest that estrogens can induce early and long-lasting changes in the monoaminergic systems (McEwen, 1999; McEwen *et al*, 2001; Mize and Alper, 2000; Österlund and Hurd, 1998). For instance, a functional desensitization of 5-HT_{1A} receptors occurs minutes after E_2 treatment (Mize and Alper, 2000) and endures at least 24 h (Raap *et al*, 2000). Therefore, a third purpose of this study was to analyze the temporal course of the antidepressant-like effect of an acute administration of the three different estrogenic compounds in the FST.

MATERIALS AND METHODS

Animals

Ovariectomized female Wistar rats (250–300 g) were housed in groups of six and maintained on a 12 h light–dark cycle (lights off at 10:00–22:00 h) in a temperature-controlled (22°C) room. The 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 our Institutional Ethics Committee.

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. Complete ovariectomy was evaluated by visual inspection of the ovaries after their extraction. After 3 weeks, animals were randomly assigned to an experimental group and behavioral studies performed.

FST

Swimming sessions were conducted by placing rats into individual glass cylinders (46 cm height × 20 cm diameter) containing 23–25°C water 30 cm deep, so that rats could not support themselves by touching the bottom with their paws. Two swimming sessions were conducted: an initial 15-min pretest 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 in heated cages for 30 min, and then returned to their home cages. Test sessions were run between 12:00 and 15:00 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 rat behaviors: (1) immobility—floating in the water without struggling, and doing only those necessary movements to keep the head above the water; (2) swimming—showing moderate active motions around in the cylinder, more than necessary to merely keep the head above water; and (3) climbing—presenting active vigorous movements with forepaws in and out of the water, usually directed against the walls.

Open-Field Test

In order to discard a possible influence of drug treatments on locomotor activity, the effect of the estrogenic compounds and antidepressant drugs in the open-field test was tested. The apparatus consisted of an opaque-Plexiglas box ($40 \times 30 \times 20\,cm^3$) with the floor divided into 12 equal squares ($11 \times 11\,cm^2$). The animals were placed in a corner of the apparatus and an observer, blind to the pharmacological treatments, registered the number of times the animal crosses squares during a 5-min session (Martínez-Mota *et al*, 1999; Estrada-Camarena *et al*, 2002).

Drugs

Estrogenic compounds: E_2, EE_2, and DES (Sigma-Aldrich, St Louis, MO, USA) were dissolved in corn oil and injected

(subcutaneous) in 0.2 ml/rat. Desipramine hydrochloride (Sigma-Aldrich, St Louis, MO, USA) and fluoxetine chlorhydrate (Eli-Lilly Company, México) were dissolved in saline solution and s.c. administered in 2.0 ml/kg.

Experimental Design

All experiments were conducted with an independent group design ($n = 10$–14 per dose). In order to analyze the possible antidepressant-like effect of the estrogenic compounds, different doses of E_2 (2.5–10.0 μg/rat), EE_2 (1.25–10 μg/rat), or DES (0.25–1.0 mg/rat) were evaluated in the FST. In addition, the behavioral profile of these estrogenic compounds was compared with that produced by the antidepressants FLX (2.5–10 mg/kg) and DMI (2.5–10.0 mg/kg). Control groups for antidepressants received saline solution and those for estrogens, corn oil. Antidepressant treatments were applied following a subacute schedule, that is, three injections administered before the FST (23.5, 5, and 1 h). For estrogenic compounds, a single dose of each steroid was administered 48 h before the FST. Latencies for drug administration were selected from previous reports (Detke *et al*, 1997; López-Rubalcava and Lucki, 2000; Martínez-Mota *et al*, 2000). Statistical analyses for each compound were performed using a one-way ANOVA test followed by Dunnett's test.

In the second part of the study, estrogens that showed antidepressant-like effects in the FST were subjected to a temporal course analysis. For this experiment, independent groups of animals (each of 6–8 rats) were tested in the FST at 1, 2, 4, 8, 12, 24, 48, 72, or 96 h after E_2 (10 μg/rat), EE_2 (5.0 μg/rat), or vehicle administration. Data were analyzed by means of a two-way ANOVA test considering treatment and time as factors, followed by Dunnett's test.

Finally, in order to detect possible unspecific effects of estrogens or antidepressant treatments, only the doses that were active in the FST were tested in independent groups ($n = 6$) in the open-field test. Statistical analyses were performed using a one-way ANOVA followed by Dunnett's test.

RESULTS

Figure 1 shows the effects of FLX (panel a) and DMI (panel b) in the FST. Both compounds significantly decreased immobility behavior ($F_{3,39} = 5.95$, $p < 0.003$ for FLX and $F_{3,45} = 2.84$, $p < 0.04$ for DMI). In addition, FLX significantly increased swimming behavior ($F_{3,39} = 6.82$, $p < 0.001$) without changing climbing behavior ($F_{3,39} = 0.36$, NS; Figure 1a). By contrast, DMI enhanced climbing ($F_{3,45} = 4.58$, $p < 0.007$) without modifying swimming behavior ($F_{3,45} = 1.38$, NS; Figure 1b).

In Figure 2a, it can be observed that E_2 significantly reduced immobility behavior ($F_{3,42} = 5.35$, $p < 0.003$) with a concomitant increase in swimming behavior ($F_{3.42} = 9.97$, $p < 0.001$) and no change in climbing ($F_{3,42} = 1.72$, NS).

On the other hand, the synthetic estrogen EE_2 at doses of 2.5 and 5 μg/rat (Figure 2b) significantly decreased

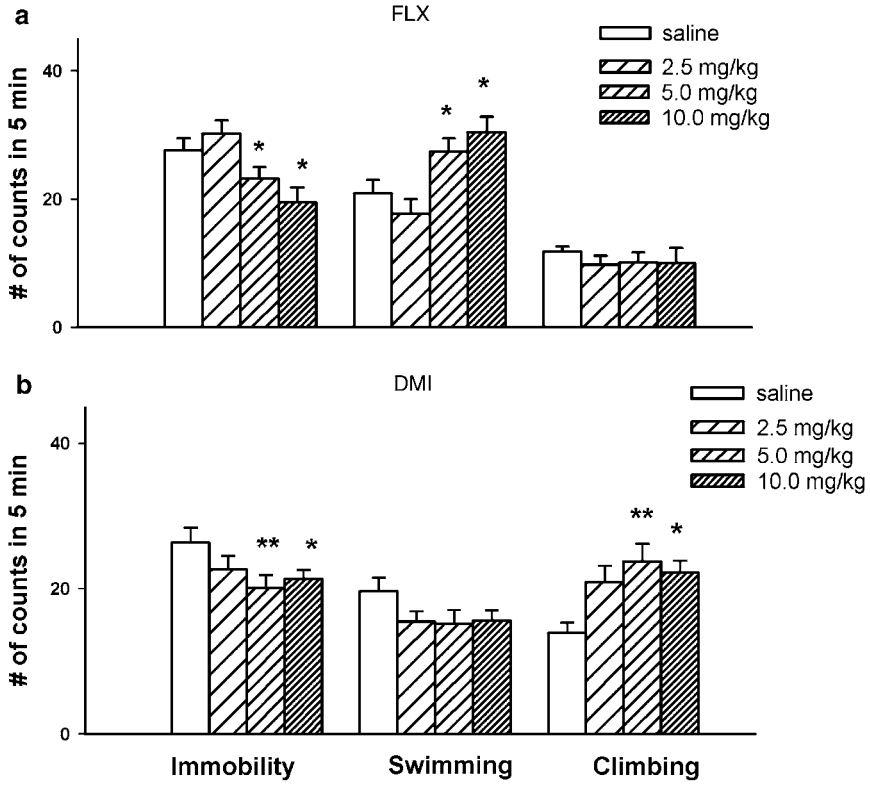

Figure 1 Behavioral effects produced by different doses of fluoxetine (FLX, −23.5, −5, −1 h, panel a, $n = 10$–12 rats per group) and desipramine (DMI, −23.5, −5, −1 h, panel b, $n = 12$–13 rats per group) in the FST. Values represent mean of counts (+1 SEM) of immobility, swimming, and climbing behaviors when sampled every 5 s during a 5-min test period. For one-way ANOVAs, see text. Comparisons *vs* control group, Dunnett's test, $^{*}p < 0.05$, $^{**}p < 0.01$.

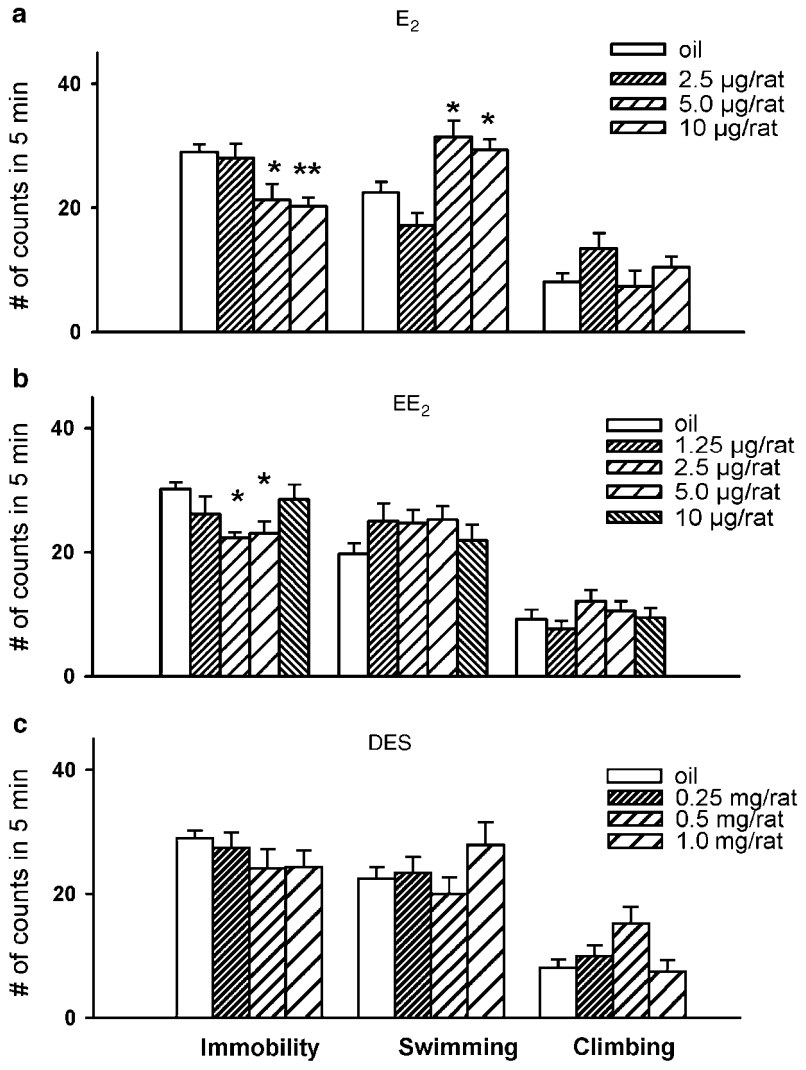

Figure 2 Behavioral effects produced by different doses of 17β-estradiol (E_2, −48 h, panel a, $n = 10$–12 rats per group), ethynil-estradiol (EE_2, −48 h, panel b, $n = 12$–14 rats per group) and diethyl-stilbestrol (DES, −48 h, panel c, $n = 10$–12 rats per group) in the FST. Values represent mean of counts (+1 SEM) of immobility, swimming, and climbing behaviors when sampled every 5 s during a 5-min test period. For one-way ANOVAs, see text. Comparisons *vs* control group, Dunnett's test, $^*p < 0.05$, $^{**}p < 0.01$.

immobility behavior ($F_{4,62} = 2.73$, $p < 0.03$) and showed a tendency towards an increase in swimming ($F_{4,62} = 0.97$, NS) without affecting climbing ($F_{4,62} = 1.09$, NS). A higher dose (10 µg/rat) shows a lack of an action on the parameters of the FST.

Finally, in marked contrast with the other estrogenic compounds, DES had no effect in the FST (Figure 2c). Thus, the immobility was not reduced after any dose of this compound ($F_{3,40} = 0.99$, NS). Similarly, the swimming behavior was unaffected ($F_{3,40} = 0.28$; NS). However, there was a tendency to increase the climbing behavior after the 0.5 mg/rat dose that was absent at the highest (1.0 mg/rat) dose level ($F_{3,40} = 3.04$, $p < 0.04$).

The effect of the three different estrogenic compounds on locomotor activity is shown in Table 1. None of the estrogens tested had an action on locomotor activity ($F_{3,22} = 0.319$, NS). FLX (10 mg/kg) and DMI (10 mg/kg), on the other hand, significantly decreased locomotor activity ($F_{2,15} = 11.73$, $p < 0.001$).

Table 1 Effect of Estrogenic Compounds and Antidepressants in the Open-Field Test

Treatment (dose)	Number of squares crossing/5 min
Oil	51.0 ± 2.79
E_2 (10 µg/rat)	52.6 ± 1.60
EE_2 (5 µg/rat)	55.1 ± 4.36
Saline	51.5 ± 4.31
DMI (10 mg/kg)	25.3 ± 4.00*
FLX (10 mg/kg)	30.0 ± 6.48*

Data are presented as mean ± SEM. For one-way ANOVAs, see text. Dunnett's test, $^*p < 0.05$ *vs* saline. E_2 = 17β-estradiol (−48 h, $n = 6$); EE_2 = ethynil-estradiol (−48 h, $n = 6$); FLX = fluoxetine (−23.5, −5, −1 h, $n = 6$); DMI = desipramine (−23.5, −5, −1 h, $n = 6$). Control groups were treated with saline solution ($n = 6$) or oil ($n = 6$).

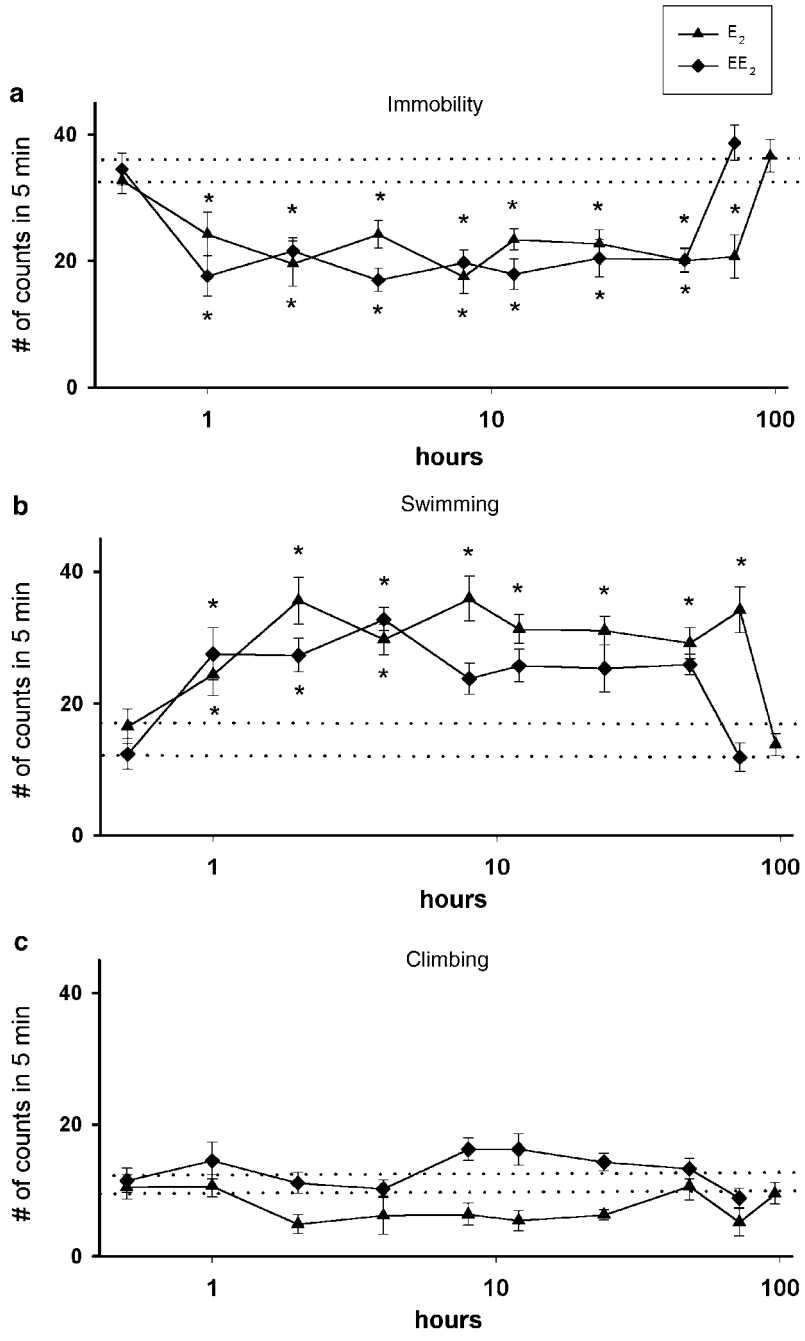

Figure 3 Time course effects for 17β-estradiol (E_2, 10 μg/rat, $n = 7$–8 rats per group) and ethynil-estradiol (EE_2, 5 μg/rat, $n = 6$–8 rats per group) in the FST. Values represent mean ± SEM of the number of counts of immobility (panel a), swimming (panel b), and climbing behaviors (panel c) when sampled every 5 s during a 5-min test period. The dotted line represents the mean ± SEM of control (oil injected, $n = 6$–7 rats) group sessions. Time scale (in hours) is represented in log. For two-way ANOVAs, see text. Comparisons *vs* control group, Dunnett's test, $^*p < 0.05$.

Since E_2 and EE_2 produced an antidepressant-like effect in the FST, the temporal course of these antidepressant-like actions was analyzed. E_2 induced a reduction in immobility (Figure 3a) and an increase in swimming behavior (Figure 3b) 1 h after its administration. These effects lasted for 72 h (Figures 3a and b). This compound did not modify the climbing behavior (Figure 3c).

EE_2, on the other hand, induced a significant reduction in immobility 1 h after its injection and this effect remained for 48 h (Figure 3a). Interestingly, this estrogen produced an increase in swimming behavior that was statistically significant from control values during the first 4 h (Figure 3b). A slight increase in climbing behavior at 8 and 12 h, which did not reach statistical significance, was observed (Figure 3c).

The two-way ANOVA test for the time course actions of E_2 and EE_2 revealed significant differences in immobility behavior for treatment ($F_{2,163} = 48.07$, $p < 0.001$), time after injection ($F_{8,163} = 5.27$, $p < 0.001$) and for the treatment × time interaction ($F_{16,163} = 3.16$, $p < 0.001$). In the

case of swimming behavior, the two-way ANOVA test showed significant differences for treatment ($F_{2,163} = 68.81$, $p < 0.001$), time ($F_{8,163} = 5.57$, $p < 0.001$) and for the treatment × time interaction ($F_{16,163} = 3.02$, $p < 0.001$). Finally, the two-way ANOVA test for the climbing behavior showed significant differences for treatment ($F_{2,163} = 23.01$, $p < 0.001$) with no significant differences for the time after injection ($F_{8,163} = 1.17$, NS) or for the treatment × time interaction ($F_{16,163} = 1.67$, NS).

DISCUSSION

In the present study it was found that both the natural and the synthetic steroidal estrogens, E_2 and EE_2, respectively, had antidepressant-like actions in the FST while the non-steroidal synthetic estrogen, DES, was inactive. The antidepressant-like effects lasted 48 h for EE_2 and 72 h for E_2.

The present study confirms the antidepressant-like effect reported for FLX and DMI. The reduction in immobility induced by FLX was accompanied by increased swimming, while that of DMI occurred with an increase in climbing behavior. The behavioral profiles observed in the present study with FLX or DMI, in ovariectomized female rats, are similar to those reported by other authors in male rats (Detke *et al*, 1995, 1997; Page *et al*, 1999; Rénéric and Lucki, 1998). Hence, the finding of Detke *et al* (1995) can also be extended to ovariectomized female rats.

Similar to the classical antidepressant treatments, E_2 and EE_2 reduced immobility behavior in the FST, an effect considered as an antidepressant-like action (Borsini, 1995; Borsini and Meli, 1988; Kitada *et al*, 1983; Porsolt *et al*, 1977, 1978). In line with the interpretations of Lucki and co-workers (Detke *et al*, 1995), the characteristic patterns of the active behaviors (swimming and climbing) in the FST would suggest that the serotonergic system participates in the antidepressant-like effect of E_2. This assumption arises from the fact that the behavioral profile induced by E_2 was similar to that observed after FLX, characterized by a decreased immobility and an increased swimming without modifying climbing (Detke *et al*, 1995; Page *et al*, 1999). However *in vitro* evidence shows that E_2 inhibits both the noradrenergic and the serotonergic reuptake sites (Ghraf *et al*, 1983; Michel *et al*, 1987). Interestingly, E_2 activity at the serotonergic transporter is superior to that exerted at the noradrenergic reuptake site (Ghraf *et al*, 1983; Michel *et al*, 1987), a difference that could underlie the behavioral profile observed. In line with this idea, the administration of the antidepressant venlafaxine, another mixed 5-HT/NA reuptake inhibitor (but more effective in inhibiting 5-HT than NA reuptake) (Béïque, 1999), also decreased immobility accompanied by an increased swimming without modifying climbing (Rénéric and Lucki, 1998). As to *in vivo* studies, although there are several reports (at least seven) that analyze the actions of E_2 on serotonergic reuptake sites, the results are inconclusive. Thus increases, decreases, and no-change on the serotonergic transporter are all reported (Attali *et al*, 1997; Cardinali and Gómez, 1977; Everitt *et al*, 1975; McQueen *et al*, 1997; Mendelson *et al*, 1993; Rehavi *et al*, 1987; Wilson *et al*, 1988). It is possible that the discrepancies could rely on variables such as the time elapsed between ovariectomy and estrogen treatment, the duration of estrogen treatment (acute *vs* chronic), the type of ligands used, and the site of the brain evaluated. It is also important to mention that none of these studies had similar conditions to the ones used in the present study (3 weeks after ovariectomy, a single steroid administration 48 h before the test). Thus, future experiments *in vivo* should be undertaken to analyze specifically the actions of E_2 on serotonin and noradrenaline reuptake sites under these experimental conditions.

Similar to E_2 the synthetic estrogen EE_2 decreased immobility behavior; however, in contrast to E_2, it showed a tendency to increase swimming. Interestingly, *in vitro* studies have shown that EE_2 inhibits both catecholaminergic and serotonergic reuptake sites with a similar potency (Ghraf *et al*, 1983; Michel *et al*, 1987). It is possible that this type of interaction could be reflected in the behavioral profile observed with this estrogen in the FST. The lack of significant increases of EE_2 on swimming behavior could be masked by the action of this steroid on the catecholaminergic system. Other authors analyzing the effect of duloxetine (a mixed 5-HT/NA reuptake inhibitor), PPT (8-methyl-2β-propanoyl-3β-(4-(-1-methylphenyl)-8-azabyciclo[3,2,1] octane)), that is a DA, NA and 5-HT reuptake inhibitor, observed a similar behavioral profile (Hemby *et al*, 1997; Rénéric and Lucki, 1998).

Interestingly, EE_2 showed a biphasic effect in the FST. Thus, low doses of this steroid (2.5 and 5 μg/rat) induced an antidepressant-like action whereas the highest dose had no effect. Clinical reports have found similar results; for example, Prange (1972) reported that low doses of EE_2 facilitate the action of the antidepressant imipramine whereas high doses had no effect. The reason for the biphasic action of EE_2 is unknown; it is possible that high doses of this compound induce unspecific actions that interfere with its antidepressant effect. Future experiments should be performed to further analyze this point.

By contrast to the other estrogenic compounds, DES did not show antidepressant-like action in this animal model of depression. *In vitro* studies have reported that DES interacts to a lesser extent with the noradrenergic system than other estrogenic compounds, such as E_2 and EE_2 (Ghraf *et al*, 1983), and that it does not affect the serotonergic reuptake sites (Chang and Chang, 1999). These data might explain its lack of effect in the FST. However, specific experiments are necessary to confirm this hypothesis.

In general, the biological actions of estrogens can be mediated through a rapid nongenomic and a delayed genomic mechanism (Cyr *et al*, 2000; McEwen, 1991, 1999; Wehling, 1997; Wise *et al*, 2001). The first mechanism is mediated by membrane receptors and second messengers (Cyr *et al*, 2000; McEwen *et al*, 2001; Nadal *et al*, 2000). The other mechanism involves the interaction with intracellular receptors that implies the activation of transcription factors (McEwen, 1991; McEwen *et al*, 2001). It is possible that both mechanisms participate in the effects of estrogens in the FST. Regarding the involvement of the rapid nongenomic mechanism, present data found an antidepressant-like effect of both E_2 and EE_2 already 1 h after their injection. *In vitro* and *in vivo* studies showed that acute injection of E_2 promotes a rapid (from 10 min to 2 h) functional desensitization of 5-HT_{1A} and 5-HT_{1B} receptors (Mize and Alper, 2000; Österlund and Hurd, 1998, 2001) and an inhibition of

serotonergic reuptake sites (Chang and Chang, 1999). Similar actions have been reported after chronic treatment with FLX and paroxetine (Li *et al*, 1996, 1997a). Thus, it is possible that the early antidepressant-like effect of estrogens involves the inhibition of reuptake sites leading to a rapid desensitization of 5-HT_{1A} and 5-HT_{1B} receptors (Chang and Chang, 1999; Mize and Alper, 2000).

In relation to the long-lasting antidepressant-like effects observed several days after a single estrogen injection, at least two nonexclusive mechanisms can be proposed. Some authors have reported a genomic effect evidenced by an increase in mRNA levels associated with an augmented 5-HT_{2A} receptor density in the frontal cortex and nucleus accumbens expressed 24–30 h after E_2 (Sumner *et al*, 1998; Sumner and Fink, 1995). Furthermore, such increase was blocked after the administration of the estrogen receptor antagonist tamoxifen (Sumner *et al*, 1998). These effects could be related to the long-lasting antidepressant-like actions of estrogens in the FST. It is notable that the 5-HT_{2A} receptor is also associated with antidepressant effects (Barnes and Sharp, 1999; Einat *et al*, 2001; Li *et al*, 1997b; Lucki *et al*, 1994; Massou *et al*, 1997; Raap and Van de Kar, 1999). The other proposed mechanism implies adaptive changes on the serotonergic and noradrenergic receptors that increase the cAMP response element binding protein (CREB) and the brain-derived neurotrophic factor (BDNF). Interestingly, the expression of CREB and BDNF is regulated by both estrogens (Murphy *et al*, 1998; Murphy and Segal, 1997) and antidepressants (Duman *et al*, 1997, 1999; Nibuya *et al*, 1996; Siuciak *et al*, 1997). Moreover, the intrahippocampal injection of BDNF induces an antidepressant-like effect in the FST that endures at least 10 days (Shirayama *et al*, 2002). Future studies should be undertaken to further examine these hypotheses.

Recently, we have found that the antidepressant-like actions of estrogens are antagonized by either WAY 100635, a specific 5-HT_{1A} blocker, or RU 58668, a selective estrogen antagonist (unpublished data). These results are in favor of the participation of both a nongenomic mechanism that primarily implies the interaction of estrogen with serotonergic receptors (see above) and a classic genomic one that considers the binding of estrogen to its intracellular receptors. Most likely, both mechanisms are involved in the antidepressant-like estrogen actions since DES, an active nonsteroidal compound that effectively interacts with the intracellular estrogen receptor (Chang and Chang, 1999; Jordan *et al*, 1985) but that almost lacks monoaminergic actions (see above), failed to induce changes in the FST.

The open-field test was designed to study the exploratory activity in rats and is commonly used in combination with the FST to discard unspecific actions of antidepressant treatments (Borsini *et al*, 1985; Borsini and Meli, 1988; Porsolt *et al*, 1978). In the present study we observed that none of the estrogen treatments produced changes in locomotor activity, indicating that the antidepressant-like activity showed by E_2 and EE_2 is not due to nonspecific changes in locomotion. By contrast, DMI and FLX reduced locomotor activity but increased active behaviors in the FST. Thus: the diminution in locomotor activity did not interfere with the expression of active behaviors indicating that the anti-immobility effect of FLX and DMI is specific in the FST.

In conclusion, E_2 and EE_2 possess long-lasting antidepressant-like effects in the FST. The different behavioral profile of these estrogens could be related with their ability to interact with the noradrenergic and serotonergic systems.

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