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Estrogens participate in the antidepressant-like effect of desipramine and fluoxetine in male rats

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Abstract

In male rats, the antidepressant-like effect of fluoxetine (FLX) and desipramine (DMI) in the forced swimming test (FST) is reduced by orchidectomy and partially restored by testosterone (T). It is unknown if this modulation of T is produced by its estrogenic metabolites. The objectives of this study were to evaluate if the aromatase inhibitor, formestane, interferes with the antidepressant-like effect of DMI and FLX in intact male rats, and to analyze if 17β-estradiol (E_2) modifies the FST and interacts with the antidepressants in orchidectomized (Orx) males. Intact males received DMI (1.25–5.0 mg/kg) and FLX (2.5–10 mg/kg) alone or in combination with formestane (17.5 mg/kg). Orx rats received E_2 (5, 10, 20 and 40 μg/rat) or the combination of E_2 [at sub-threshold (5 μg/rat) and optimal (10 μg/rat) doses] plus sub-effective doses of DMI (2.5 mg/kg) or FLX (10 mg/kg). Serum testosterone and estradiol levels were measured in intact-control and -formestane treated animals as well as in castrated males replaced with various doses of E_2. Formestane in intact males lacked of an action in the FST, but cancelled the antidepressant-like effect of DMI and FLX. E_2 at the supra-physiological doses of 10 and 20 μg/rat produced antidepressant-like effects. E_2 at 5 μg/rat (that re-established the levels of this hormone to physiological levels) and at 10 μg/rat restored the antidepressant-like action of DMI and FLX in Orx rats. It was concluded that estrogens participate in the antidepressant-like effect of DMI and FLX in the FST.

Keywords: Aromatase inhibition; Estrogens; 17β-Estradiol; Desipramine; Fluoxetine; Forced swimming test; Antidepressants; Male rats

1. Introduction

Several lines of evidences suggest that the main androgen in males, testosterone (T), participates in mood, mental state and behavior. A clear relationship between T and these alterations is shown in men with low levels of androgens, i.e. hypogonadal patients, which exhibit a cognitive deficit and an increased risk to suffer depression (Zitzmann, 2006). In animal models of psychiatric disorders it has been established that several effects attributable to T are mediated by its metabolites. Thus, 5α-dihydrotestosterone (DHT) and 3α,5α-androstanediol (3α-diol) decrease anxiety-like behavior, participate in cognitive performance (Edinger and Frye, 2005; Edinger et al., 2004) and mediate the positive hedonic effect of T (Rosellini et al., 2001; Frye et al., 2001). Interestingly, recent data suggest that some effects of 3α-diol on learning in males are mediated by the β-estrogen receptor (Edinger and Frye, 2007). Estrogens, which are synthesized from androgens by aromatase, mediate organizational functions of T, such as brain differentiation, and modulate the expression of copulatory behavior in rodents (Sharpe, 1998). In female rats, 17β-estradiol (E_2) produces antidepressant-and anxiolytic-like effects (Estrada-Camarena et al., 2003; Shors and Leuner, 2003; Walf and Frye, 2005) but in males its role in depressive-like behaviors has not been analyzed.

The function of the serotonergic (5-HT) and noradrenergic (NA) systems, which are importantly altered in mood disorders, is modulated by T and estrogens. Accordingly, orchidectomy reduces the levels of: (a) 5-HT in hypothalamus and hippocampus,

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(b) 5-HT transporter mRNA in the dorsal raphe nucleus and (c) 5-HT transporter binding sites in the dorsal raphe and arcuate nuclei. Interestingly, estrogens but not androgens revert these changes (Bitar et al., 1991; Fink et al., 1999; McQueen et al., 1999). In addition, orchidectomy reduces 5-HT_{2A} receptor mRNA and protein and this effect is reversed by T and estrogens (Sumner and Fink, 1998). Regarding the NA system, orchidectomy increases the number of [^{3}H]nisoxetine binding sites (an evidence for increased NA transporter number) in the rat frontal cortex (Shang et al., 1999), unfortunately the role of T or estrogens in this effect has not been explored. Even though, these evidences, taken together, point towards a main role of the testicular hormones: T and its estrogenic metabolites in the regulation of the monoaminergic transmission that participates in depression.

In this respect, several authors have reported a relationship between the presence of depressive disorders in young-and middle-aged men and a decrease of total or free T plasmatic concentrations (Schweiger et al., 1999; Wagner et al., 1996). This association is more evident in depressed hypogonadal patients that are insensitive to selective serotonin reuptake inhibitors (SSRIs, i.e. fluoxetine or FLX), and in which T restitution recovers the antidepressant action of SSRIs (Seidman and Rabkin, 1998; Seidman et al., 2005). Interestingly, in an animal model of depression-the rat forced swimming test (FST)—we observed that orchidectomy attenuated the antidepressant-like effect of desipramine (DMI) and FLX and T supplementation re-established the action of DMI but not that of FLX (Martínez-Mota and Fernández-Guasti, 2004). From these results it was concluded that T participates in the antidepressant action of DMI, while the effect of FLX seems to depend upon another endocrine mechanism.

Thus, the main aim of the present study was to analyze the role of estrogens in the antidepressant-like action of DMI and FLX in male rats, in the FST. Two experimental approaches were used: in the first, the effect of DMI and FLX was assessed in intact (non Orx) males with decreased brain levels of estrogens due to treatment with the aromatase inhibitor 4-hydroxy-androstenedione (formestane). In the second, Orx rats were given various doses of E_2 to investigate the antidepressant-like effect of this hormone as a single treatment or in combination with DMI and FLX. Previous studies have demonstrated that estrogens increase the antidepressant-like effects of DMI and FLX in ovariectomized female rats (Estrada-Camarena et al., 2004). To assess the effects of treatment with formestane in intact male rats and various doses of E_2 in orchidectomized animals, the serum levels of estradiol and testosterone were measured by radioimmunoassay.

2. Materials and methods

2.1. Animals

Male adult Wistar rats weighing 250–300 g were used in this study. All animals were obtained from the vivarium of the *Instituto Nacional de Psiquiatría*; rats were housed, five per cage, in a room under inverted light/dark cycle conditions (12/12 h; lights on at 2200 h) with *ad libitum* access to water and Purina Rat Chow throughout the experiments. The local ethical committee for animal use approved the protocol for these experiments. Animal management was done according to the general principles of laboratory animal care (NIH publication 85-23, 1985).

2.2. Surgery

Rats were bilaterally Orx under 2,2,2-tribromoethanol (Fluka, 100 mg/kg i.p., dissolved in 0.9% saline solution) anesthesia and aseptic conditions. Briefly, a single midline incision was made in the low abdominal area to expose the testes; the vas deferens were bilaterally ligated and the testes removed. The muscle and skin were sutured and the ventral area cleaned with an antiseptic solution. Afterwards, the rats were returned to their home cages to recover for at least three weeks before the experiments or sacrifice.

2.3. Drugs

DMI hydrochloride and FLX hydrochloride (Sigma Chemicals Co., MO) were dissolved in 0.9% saline solution, freshly prepared before each experiment and injected in a volume of 0.2 ml/100 g weight. E_2 (Sigma Chemicals Co., MO) was dissolved in corn oil with two drops of dichloromethane (Sigma Chemicals Co., MO) and injected in a volume of 0.2 ml/rat. Formestane (Sigma Chemicals Co., MO) was suspended in a mixture of propylene glycol and corn oil 1:1 and injected in a volume of 0.1 ml/100 g weight.

2.4. Forced swimming test (FST)

The FST was selected as an animal model to study experimental depression, since it is sensitive and selective to antidepressant drugs (Porsolt et al., 1977). Additionally, this model reveals behavioral changes associated to hormonal variations, i.e. the rat estrous cycle, or administration of exogenous ovarian hormones (Contreras et al., 1998; Estrada-Camarena et al., 2003; Martínez-Mota et al., 1999; Rachmann et al., 1998). Furthermore, it has been suggested that the FST reveals the participation of 5-HT or NA neurotransmission in the antidepressant-like effect of various compounds. Thus, in this test, the main effect of antidepressants consists in a reduction of immobility accompanied by an increase in either of the two active behaviors: swimming denoting 5-HT participation, or climbing revealing NA involvement (Detke et al., 1995).

Rats were tested 3–4 h after the beginning of the dark phase (13:00–14:00 h) in the modified FST described by Detke et al. (1995, 1997). The rats were individually placed in a glass cylinder (46 cm height × 20 cm diameter) containing 30 cm of water at 23 (±2) °C. Two swimming sessions were conducted: the first was a 15-min pre-test session followed, 24 h later, by a 5-min test. At the end of each session, animals were removed from the jars, dried with tissue paper and placed to recover in a warm cage (23 ± 2 °C) during 15 min. The 5-min test was videotaped and later scored by two observers unaware of the treatments applied.

A time-sampling technique was employed to score, every 5 s, one of the following behaviors: a) immobility, defined as the minimum movements done by animals to keep their nostril above the water (i.e. floating); b) swimming, active motions made by animals that result in movements within the pool (i.e. moving around the jar and diving); c) climbing, defined as strong movements executed with forepaws in and out of the water usually against the walls (Detke et al., 1995, 1997). Thus, in a 5-min test a total of 60 counts, including immobility, swimming or climbing were obtained. The frequency of each behavior was expressed as the mean ± SE.

2.5. Ambulation test

An ambulation test was conducted in order to discard unspecific drugs actions (i.e. an increase of locomotor activity) on the behavioral changes observed in the FST. The rats were placed in a Plexiglas cage with the floor (33 × 44 × 20 cm) divided in squares (11 × 11 cm); the number of squares crossed by the rats (counts) was counted in a 5-min test and the results were expressed as mean ± SE.

2.6. Experimental series

2.6.1. Effect of DMI and FLX in intact male rats

This experiment was conducted to establish the effective doses of DMI and FLX in intact males subjected to the FST. Independent groups of rats were treated with DMI (1.25, 2.5 and 5 mg/kg, n = 7–12 per group) or FLX (2.5, 5 and 10 mg/kg, n = 9–10 per group) in a sub-chronic schedule that consisted of three s.c. injections between the pre-test and test sessions (23.5, 5 and 1 h before the 5-min swimming test). The control groups (n = 10–12 rats per group) received three s.c. injections of 0.9% saline in the same schedule used for the drugs. DMI and FLX doses given to intact male rats and their schedule of administration were chosen from previous experiments that demonstrate clear antidepressant-like actions (Detke et al., 1995; Lopez-Rubalcava and Lucki, 2000; Martínez-Mota and Fernández-Guasti, 2004). It is worth mentioning that the subacute schedule here used for DMI and FLX produces an analogous antidepressant-like response to that induced by a chronic administration (Detke et al., 1997).

2.6.2. Effect of formestane on the actions of DMI and FLX

In this experiment the participation of endogenous estrogens in the antidepressants' effect was evaluated in intact males subjected to the FST. Independent groups of rats (6–9 per group) were assigned as follows: a) control, animals treated with vehicle (1:1 corn oil plus propyleneglycol and 0.9% saline solution); b) 17.5 mg/kg formestane; c) 5 mg/kg DMI; d) 10 mg/kg FLX; e) 17.5 mg/kg formestane plus 5 mg/kg DMI and f) 17.5 mg/kg formestane plus 10 mg/kg FLX. The antidepressant drugs and 0.9% saline solution were injected as described in experiment 1; effective doses of DMI and FLX were selected of experiment 1 with intact males. Formestane (or its vehicle) was s.c. injected for four days twice daily, with the last injection applied 5 h after the pre-test session. Formestane is a type I steroidal compound that inactivates the aromatase enzyme complex; this compound is an androgen substrate analogue, that binds competitively but irreversibly to the enzyme (Miller, 2003). Previously it has been reported that this dose and schedule of formestane administration reduces the brain aromatase activity in intact male rats to less than 10 fmol/mg protein/h *versus* 175 fmol/mg protein/h in vehicle-treated rats (Yuan et al., 1995).

2.6.3. Effect of E_2 in Orx male rats

In this experiment the effect of physiological and pharmacological doses of E_2 was evaluated in Orx male rats subjected to the FST. Rats were Orx to control endogenous estrogen levels and aromatase activity, which is stimulated by gonadal steroids (Roselli, 1991; Roselli et al., 1996). Independent groups of rats were treated with a single s.c. dose of E_2 (5, 10, 20 or 40 μg/rat, n = 9–11 per group) 48 h before the test. The control group was subcutaneously injected with vehicle (corn oil). The doses of E_2 and the schedule of administration were chosen considering their effectiveness to produce antidepressant-like actions in female rats (Estrada-Camarena et al., 2003; Walf and Frye, 2005).

2.6.4. Effect of E_2 plus DMI or FLX in Orx rats

This experiment was conducted to study the possible interaction between E_2, at physiological and pharmacological doses, and the antidepressant drugs DMI or FLX. For this purpose, two doses of E_2 were used: 5 or 10 μg/rat. DMI or FLX were administered in at ineffective doses in Orx male rats (Martínez-Mota and Fernández-Guasti, 2004). Independent groups of Orx rats were assigned to the following treatments: a) control (corn oil plus 0.9% saline, n = 10), 2.5 mg/kg DMI (corn oil plus DMI, n = 10) or 10 mg/kg FLX (corn oil plus FLX, n = 8); b) 5 μg/rat E_2 (E_2 plus 0.9% saline, n = 9) or 10 μg/rat E_2 (E_2 plus 0.9% saline, n = 10); c) 5 μg/rat E_2 plus 2.5 mg/kg DMI (n = 9); d) 10 μg/rat E_2 plus 2.5 mg/kg DMI (n = 12); e) 5 μg/rat E_2 plus 10 mg/kg FLX (n = 8); f) 10 μg/rat E_2 plus 10 mg/kg FLX (n = 12). E_2 and corn oil were s.c. injected 48 h before the 5-min swimming test. DMI, FLX and 0.9% saline were s.c. administered in the sub-chronic schedule previously described in experiment 1.

2.6.5. Effect of treatments on locomotor activity

All groups were tested for locomotor activity 5 min before the FST session. Only the ambulatory activity of treatments that reduced immobility in the FST is reported.

2.6.6. Determinations of estradiol and testosterone in serum

Intact or castrated male rats were sacrificed by decapitation to obtain trunk blood and to analyze the serum concentrations of estradiol and testosterone. The groups sacrificed were: intact males treated with vehicle (corn oil), intact males treated with formestane (17.5 mg/kg), Orx males treated with vehicle; and Orx males treated with E_2 at 5, 10, 20 or 40 μg/rat. Oil and E_2 were subcutaneously administered 48 h before sacrifice. Formestane (or its vehicle) was subcutaneously injected for four days twice daily, and rats sacrificed 19 h after the last injection.

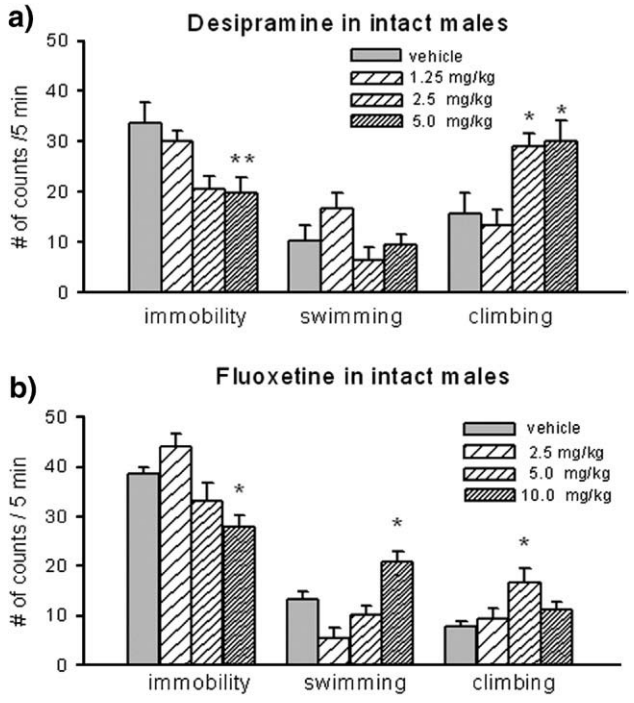

Fig. 1. Effect of DMI and FLX in intact male rats subjected to the FST. Data is shown as mean±SE. Dunnett's test: *$p<0.05$; **$p<0.01$ *versus* vehicle-treated control group.

Total plasma testosterone or estradiol concentrations were measured by radioimmunoassay using commercial kits (TKTT1 or TKE21, Diagnostic Product Corporation, for testosterone and estradiol, respectively). The procedure used antibody-coated tubes in which ^{125}I-labelled testosterone or estradiol competed with free testosterone or estradiol, respectively, in the sample for antibody sites. After incubation, separation of bound testosterone or estradiol was achieved by decanting. The tubes were then counted in a gamma counter, the counts being inversely related to the amount of testosterone or estradiol present in the serum. Testosterone (in ng/ml) or estradiol (in pg/ml) total quantity was determined by comparing counts to a calibration curve. The specific activity was 4 μci. The sensitivity limit for testosterone was 0.0045 ng/ml and for estradiol 0.13 pg/ml. The inter-assay and intra-assay variabilities for testosterone were 7.89 and 7.23%, respectively, and for estradiol 8.41 and 7.56%, respectively.

2.7. Statistics

Treatment effects on the FST, ambulation and sex steroid levels were analyzed with one-way ANOVAs. When the variance analysis attained at least a $p<0.05$ it was followed by a post hoc test. The Dunnett's test was used when comparing against a control group and the Tukey's test was used for multiple comparisons.

3. Results

3.1. Effect of DMI and FLX in intact male rats

In intact male rats DMI (Fig. 1, panel a) produced a statistically significant reduction in immobility ($F_{3,39}=3.42$, $p=0.02$) together with an increase in swimming ($F_{3,39}=4.24$, $p=0.01$) and climbing ($F_{3,39}=5.67$, $p=0.003$). The post hoc test revealed that the antidepressant-like effect of DMI (i.e. 40% reduced immobility with respect to the control group) was produced at the dose of 5 mg/kg although an increase in climbing behavior was observed from 2.5 mg/kg onwards. Statistical significance was found for swimming behavior due to a difference between the lowest and middle doses of DMI ($p<0.05$). Treatment of intact rats with FLX (Fig. 1, panel b) induced a reduction in immobility ($F_{3,39}=8.22$, $p<0.001$) and an increase in swimming ($F_{3,39}=11.78$, $p<0.001$) and climbing ($F_{3,39}=3.95$, $p=0.01$). The highest dose of FLX produced a reduction in immobility behavior (27% *versus* its respective control) accompanied by increased swimming; whereas the dose of 5 mg/kg increased climbing behavior, but this effect was not accompanied by changes in immobility.

3.2. Effect of formestane on DMI and FLX's actions

Treatment with formestane did not modify the behavior in intact male rats in the FST (Fig. 2). This result is in line with the lack of action of orchidectomy in this test (Martínez-Mota and Fernández-Guasti, 2004). However, formestane completely abolished the antidepressant-like action of DMI (Fig. 2, panel a), observed by the blockade of DMI's effect on immobility ($F_{3,29}=12.79$, $p<0.001$) and climbing ($F_{3,29}=14.31$, $p<0.001$). DMI did not produce changes in swimming behavior either alone or plus formestane ($F_{3,29}=2.06$, $p=0.13$). In a similar manner, formestane effectively cancelled the antidepressant-like effect of FLX (Fig. 2, panel b), revealed as the blockade of FLX action on immobility ($F_{3,31}=49.24$, $p<0.001$) and swimming ($F_{3,31}=41.04$, $p<0.001$). Neither FLX nor

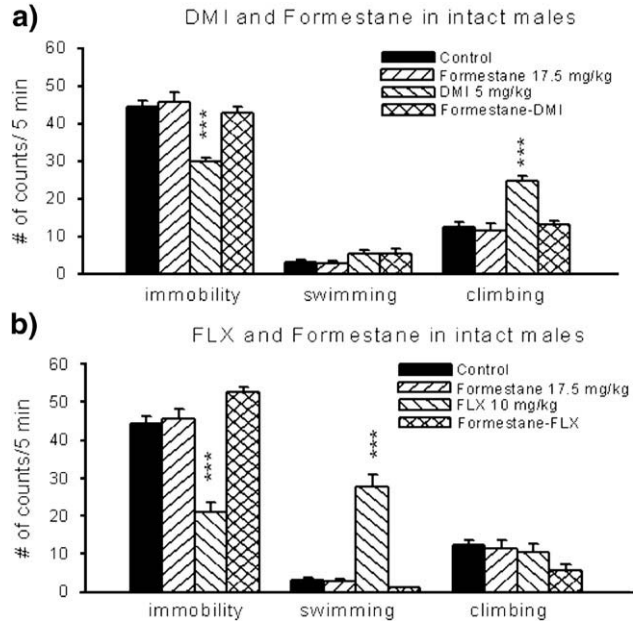

Fig. 2. Effect of formestane (17.5 mg/kg) on antidepressant-like actions of DMI and FLX in intact males subjected to the FST. Data is shown as mean±SE. Dunnett's test: ***$p<0.001$ *versus* vehicle-treated control group.

formestane produced changes in climbing behavior ($F_{3,31}=2.59$, $p=0.07$).

3.3. Effect of E_2 in Orx male rats

Treatment with a single injection of E_2 (Fig. 3) produced an anti-immobility effect in Orx male rats ($F_{4,49}=5.77$, $p<0.001$) and an increase in active behaviors (swimming $F_{4,49}=4.31$, $p=0.005$; climbing $F_{4,49}=5.95$, $p<0.001$). The post hoc test showed that reduced immobility was observed at 10 and 20 μg/rat (31 and 24%, *versus* control group, respectively). Different E_2 doses produced statistically significant increases in active behaviors: 10 μg/rat increased climbing behavior, whereas 20 μg/rat increased swimming. A low physiological dose of E_2: 5 μg/rat, or a very high dose of this steroid: 40 μg/rat lacked of an action in the FST.

3.4. Effect of E_2 plus DMI or FLX in Orx rats

As previously reported, DMI at 2.5 mg/kg and FLX at 10 mg/kg were completely ineffective in producing antidepressant-like actions in Orx males (Martínez-Mota and Fernández-Guasti, 2004). Treatment with E_2 in combination with DMI (Fig. 4, panel a) statistically modified immobility ($F_{5,60}=4.79$, $p=0.001$), swimming ($F_{5,60}=3.26$, $p=0.01$) and climbing ($F_{5,60}=4.16$, $p=0.003$) behaviors. Post hoc comparisons revealed that a sub-optimal dose of E_2 (5 μg/rat) in the FST permitted the antidepressant-like effect of DMI by reducing immobility (at around 40%) and increasing climbing. A higher dose of E_2 did not further reduce immobility or increase climbing when combined with DMI. In addition, reduced swimming behavior was observed with the optimal dose of E_2 (10 g/rat) plus DMI, in comparison to DMI alone.

Treatment with E_2 in combination with FLX (Fig. 4, panel b) also produced changes on immobility ($F_{5,57}=19.46$, $p<0.001$) and climbing ($F_{5,57}=13.46$, $p<0.001$), without variations in swimming ($F_{5,57}=1.89$, $p=0.11$). Post hoc tests revealed that the sub-optimal dose of E_2 (5 μg/rat) in combination with FLX produced a significant increase in immobility behavior. In contrast, the optimal dose of E_2 in the FST (that reduces the immobility behavior in 31%) in combination with FLX reduced immobility behavior (by 46%) and increased climbing behavior in comparison to the vehicle-treated group.

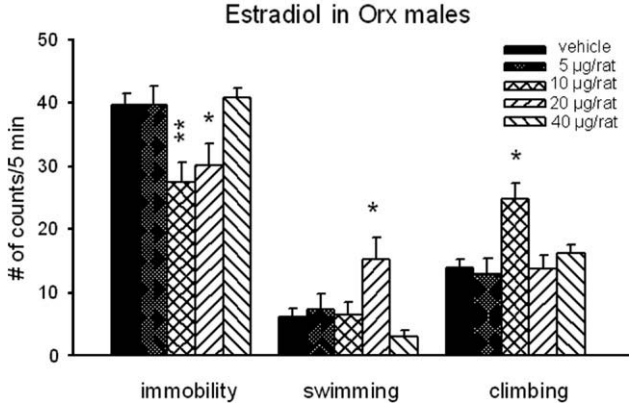

Fig. 3. Effect of different E_2 doses in orchidectomized rats subjected to the FST. Data is shown as mean±SE. Dunnett's test: *$p<0.05$; **$p<0.01$ *versus* vehicle-treated control group.

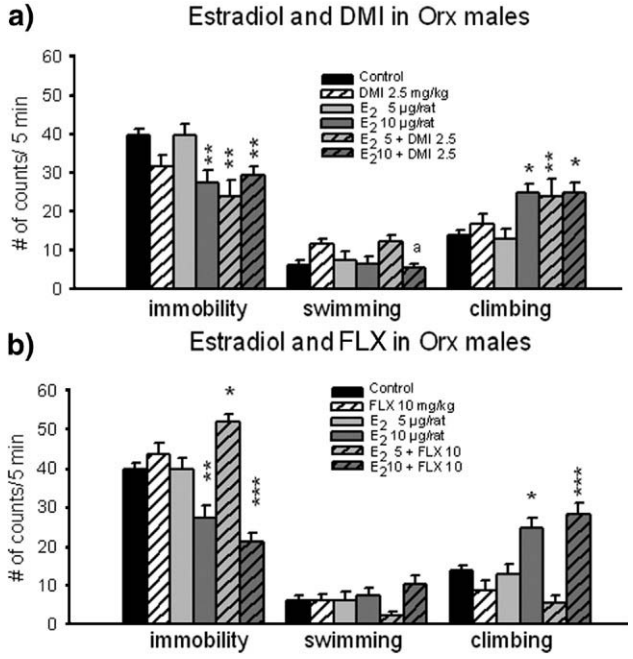

Fig. 4. Effect of the combined administration of E_2 and DMI or FLX in orchidectomized rats subjected to the FST. Data is shown as mean±SE. Tukey's test: *$p<0.05$, **$p<0.01$, ***$p<0.001$ *versus* vehicle-treated control group; [a] $p<0.05$ *versus* DMI-treated group.

3.5. Effect of the treatments on the locomotor activity

Effective DMI and FLX doses (which reduce immobility behavior in the FST) significantly reduced ambulation of intact male rats ($F_{2,30}=6.95$, $p=0.007$, see Table 1). In Orx males (see Table 2) these treatments also significantly reduced the ambulatory activity ($F_{9,96}=6.69$, $p<0.001$). Post hoc comparisons revealed a reduction in the number of counts with 2.5 mg/kg DMI alone and DMI or FLX (10 mg/kg) in combination with both doses of E_2.

3.6. Determinations of estradiol and testosterone in serum

The analyses showed changes in the plasmatic levels of estradiol ($F_{6,49}=11.57$, $p<0.001$) and testosterone ($F_{6,49}=49.76$, $p<0.001$) by effect of the treatments (see Table 3). Orchidectomy produced a significant reduction in testosterone concentration and a mild effect on estradiol levels that did not reach statistical

Table 1
Effect of DMI and FLX on locomotor activity of intact male rats

Treatments	N	Number of counts/5 min
Control (saline)	10	53.00±6.23
DMI 5 mg/kg	11	34.16±2.35*
FLX 10 mg/kg	10	31.66±3.02*

Data show mean±SE. Dunnett's test: *$p<0.05$ *versus* the vehicle-treated control group.

Table 2
Effect of various treatments on locomotor activity of orchidectomized male rats

Treatments	*N*	Number of counts/5 min
Oil-saline	10	49.83±6.35
E_2 10 μg/rat	10	57.66±6.10
E_2 20 μg/rat	9	38.16±6.24
E_2 40 μg/rat	9	32.75±3.20
DMI 2.5 mg/kg	10	23.85±2.65*
FLX 10 mg/kg	8	32.71±3.94
E_2 5 μg/rat+DMI 2.5 mg/kg	9	29.33±2.74*
E_2 10 μg/rat+DMI 2.5 mg/kg	12	29.85±3.86*
E_2 5 μg/rat+FLX 10 mg/kg	8	30.85±3.81*
E_2 10 μg/rat+FLX 10 mg/kg	12	28.00±3.77*

Data show mean±SE. Dunnett's test: *$p<0.05$ *versus* the vehicle-treated control group.

significance. Formestane lacked of an effect on testosterone and estradiol serum levels, although this treatment has been reported to drastically reduce the brain estrogen levels (Yuan et al., 1995). Treatment with 5 μg/rat of E_2 to Orx rats restored the estradiol's concentration to a physiological level. The administration of higher doses of this steroid induced supra-physiological concentrations. E_2 treatment did not modify the testosterone levels which always were maintained under the physiological concentration.

4. Discussion

The present results support the idea that estrogens participate in the antidepressant-like actions of DMI and FLX in male rats. Thus, when the production of brain estrogens was limited by formestane (Yuan et al., 1995), the antidepressant-like action of DMI and FLX was cancelled. Additionally, DMI and FLX recovered their antidepressant-like actions in Orx males when combined with E_2 (5 or 10 μg/rat, respectively). Moreover, the administration of pharmacological doses of E_2 (10 and 20 μg/rat) to Orx males produced an antidepressant-like effect.

It has been suggested that male gonadal hormones participate in the regulation of affective-like behaviors (Bitran et al., 1993; Bernardi et al., 1989; Frye and Edinger, 2004; Edinger and Frye, 2005). Thus, in animal models of anxiety such as the open field-, plus maze-, and burying behavior-tests, castration increased anxiety-like behaviors which were reduced by androgens administration (Frye and Edinger, 2004; Edinger and Frye, 2005; Fernández-Guasti and Martínez-Mota, 2003). In contrast, orchidectomy, T supplementation, or formestane treatment did not modify depressive-like behaviors in the FST (Martínez-Mota and Fernández-Guasti, 2004, present results), suggesting that changes in plasmatic or brain concentrations of testicular steroids do not affect the expression of depressive-like behaviors in the FST. These discrepancies imply that the animal models of anxiety and depression possess a differential sensitivity to testicular secretions. Although this conclusion seems plausible from a physiological perspective, it does not appear to extend to the effect of psychoactive drugs. Thus, in the FST orchidectomy reduced the antidepressant-like effect of DMI and FLX (Martínez-Mota and Fernández-Guasti, 2004) and increased the animals' reactivity to diazepam (Fernández-Guasti and Martínez-Mota, 2003). These data, taken together, indicate that behavioral changes in affective-like behaviors may be related to the gonadal endocrine milieu both in males and females (Contreras et al., 1998; Frye and Walf, 2002), particularly revealing interactions between androgens–or their metabolites–and the neurotransmitter systems that are target of these psychoactive drugs.

Previously we proposed that endogenous estrogens in males may be participating in the antidepressant-like effect of DMI and FLX in the FST (Martínez-Mota and Fernández-Guasti, 2004). Accordingly, the blockade of brain aromatase activity by formestane, completely interfered with the antidepressant-like actions of these drugs in intact male rats (present results). Formestane is a steroidal compound that reduces the synthesis of estrogens from androgens by inhibiting aromatase activity (Dowsett, 1999). In this study formestane did not modify estrogen and testosterone plasma concentrations, however, this same treatment has been reported to significantly reduce the brain aromatase activity: lesser than 10 fmol/mg protein/h *versus* 175 fmol/mg protein/h in vehicle-treated rats (Yuan et al., 1995). In addition, formestane does not appear to modify the metabolism of antidepressant drugs (Caccia, 1998); suggesting that the reduction of brain estrogen levels is responsible for the blockade of the action of DMI and FLX.

Estrogens have been involved in depression (Studd and Panay, 2004) and reported to produce antidepressant-like actions in women and ovariectomized female rats (Rachmann et al., 1998; Estrada-Camarena et al., 2003; Shors and Leuner, 2003; Walf and Frye, 2005). In the present study E_2 at pharmacological doses, and subcutaneously administered 48 h before the test, also produced antidepressant-like effects in Orx male rats. The effect of this treatment with E_2 on behavioral despair may be related to the action of this steroid on the regulation of the hypothalamus–pituitary–adrenal (HPA) axis. Stressors activate the HPA axis by promoting an increase in plasma corticosterone levels, which are associated with increased anxiety-and depressive-like behaviors (Kirby and Lucki, 1998; Kirby et al., 1997; Walf and Frye, 2005). Moreover, stressed Orx rats show a higher increase in corticosterone plasma levels compared to intact animals (Viau and Meaney, 1996). Testosterone, 3β-diol and β-estrogen receptor agonists reduce HPA axis activation (Lund et al., 2006) suggesting that the antidepressant-like effects of E_2 in Orx male rats may be mediated by a blunted HPA axis activation. The

Table 3
Concentration of estradiol and testosterone in serum, of intact or castrated male rats treated with formestane or E_2, respectively

	Estradiol pg/ml	Testosterone ng/ml	*N*
Intact-vehicle	12.97±2.18	3.65±0.45	7
Intact-formestane	22.03±5.00	2.63±0.35	7
Orx-Vehicle	7.37±1.25	0.12±0.06*	9
Orx-E_2 5 μg/rat	18.37±3.88	0.09±0.04*	8
Orx-E_2 10 μg/rat	85.06±11.22*	0.01±0.004*	6
Orx-E_2 20 μg/rat	431.93±114.20*	0.02±0.007*	7
Orx-E_2 40 μg/rat	411.18±115.16*	0.09±0.06*	6

Results of Dunnett's test: * $p<0.05$ *versus* vehicle-intact group.

time course relationship between E_2 injection and FST favors this interpretation: thus, anxiolytic drugs applied before the pre-test session (but not between the pre-test and the test) reduce HPA axis activity elicited by the pre-test and produce antidepressant-like effects (De Pablo et al., 1991). In addition, E_2 administered s.c. 48 h before anxiety and depression tests produces anxiolytic-and antidepressant-like behaviors in ovariectomized females by interacting with the HPA axis (Frye and Walf, 2004; Walf and Frye, 2005). Therefore, it is possible that the antidepressant-like effect of E_2 was mediated by its anxiolytic-like effect.

In the present study E_2 at pharmacological doses (10 and 20 μg/rat, s.c.) produced antidepressant-like actions in Orx males. This effect of E_2 has also been reported for ovariectomized female rats; which respond to doses in a range from 5 to 20 μg/rat in a similar schedule (s.c. 48 h before the test) (Estrada-Camarena et al., 2003; Walf and Frye, 2005) suggesting that females possess a higher sensitivity to E_2 than males. Interestingly, neither sex responded to higher E_2 doses: 40 μg/rat for males (present results) and 50 μg/rat for females (Walf and Frye, 2005) possibly because very high plasma concentrations of estrogens were reached. In line with this interpretation, estradiol at 5 μg/rat, but not at 1, 10 or 20 μg/rat, effectively restored the compulsive-like action of the serotonergic agonist, 8-hydroxy-2-(di-*n*-propyl amino) tetralin (8-OH-DPAT) in castrated males (Reyes-Serrano et al., submitted), and moderated levels of estrogens (but not very low or very high levels of the steroid) produced an enhancement of cognitive performance in females (Shors and Leuner, 2003), suggesting that the antidepressant-like effect of E_2 in male rats depends on the dosage.

The reduced immobility produced by E_2 was accompanied by an increase in both active behaviors; however, they were differentially affected by the E_2 dose: 10 μg/rat increased climbing, while 20 μg/rat increased swimming. An increase in swimming reveals activation of the 5-HT system, while an increase in climbing denotes activation of NA neurotransmission (Detke et al., 1995, 1997). Accordingly, the 5-HT_{1A} receptor seems to participate in the antidepressant-like effect E_2 in female rats given in a similar treatment (10 μg/rat, s.c., 48 h before the test). This idea is supported by a synergism between E_2 and the 5-HT_{1A} agonist, 8-OH-DPAT and by the blockade of the E_2 effect by the 5-HT_{1A} receptor antagonist, WAY 100635 (Estrada-Camarena et al., 2006). Furthermore, the α_2-adrenergic antagonist (idazoxan) effectively blocked the antidepressant-like action of ethynil-estradiol (Estrada-Camarena et al., unpublished results). Additionally, estrogens elevate the mRNA levels of both tyrosine hydroxylase and dopamine beta-hydroxylase in various brain areas (Serova et al., 2002; Maharjan et al., 2005). This evidence suggests that both the 5-HT and NA systems may be implicated in the antidepressant-like effect of estrogens in males; however, the differential dose-effects on active behaviors deserve future experiments.

In agreement with our results, E_2 treatment facilitated the antidepressant-like action of DMI and FLX (present results), whereas T only facilitated DMI effect (Martínez-Mota and Fernández-Guasti, 2004). This difference could be due to the action that E_2 and T have on the 5-HT and NA systems, which FLX and DMI target. Thus, orchidectomy decreases all monoamines in brain structures reported to participate in depression-such as hypothalamus, hippocampus and olfactory bulb (Cornwell-Jones and Marasco, 1980; Putnam et al., 2001; Bitar et al., 1991). T supplementation re-established the levels of these neurotransmitters, but chronic E_2 was more effective than T in restoring 5-HT levels (Bitar et al., 1991). Orchidectomy also reduced the level of 5-HT transporter mRNA in the dorsal raphe and its binding sites in the arcuate nucleus and median raphe; all of which are restored by acute administration of estradiol benzoate (30 μg/kg, s.c), but not by androgens (Fink et al., 1999; McQueen et al., 1999). All these data suggest that estrogens are the crucial hormones in the antidepressant action of compounds that act on the serotonergic system like FLX and CMI (Martínez-Mota and Fernández-Guasti, 2004).

In Orx males, a dose of E_2 (5 μg/rat) that produces physiological levels of estrogens permitted the antidepressant-like action of DMI (Martínez-Mota and Fernández-Guasti, 2004). Increasing the dose of E_2 (to 10 μg/rat) did not further modify the antidepressant-like effect of DMI. All reductions in immobility produced by DMI in combination with E_2 were accompanied by an increase in climbing behavior, stressing the role of the NA system in the actions of this compound. The effects of estrogen in combination with FLX were more complex: 5 μg/rat of E_2 reduced the effectiveness of FLX (10 mg/kg) in inducing a pro-depressive effect; however, E_2 at 10 μg/rat (that induced supra-physiological levels of this steroid) facilitated the antidepressant-like effect of FLX, together with an increased climbing. The different E_2 doses that facilitate the antidepressant-like actions of DMI or FLX and the biphasic effect of E_2 combined with FLX, suggest that the 5-HT system is more sensitive than the NA system to variations in the gonadal endocrine milieu, particularly to estrogen concentrations. This suggestion is supported by differential effects of orchidectomy on the actions of the antidepressants: a blunted DMI response compared with a complete absence to CMI and FLX action (Martínez-Mota and Fernández-Guasti, 2004).

Notwithstanding the main role of 5-HT and NA systems in the antidepressant-like actions of FLX and DMI, respectively, other brain systems may participate in the behavioral effects produced by these drugs. Thus, FLX stimulates the synthesis of neurosteroids, i.e. 3α-5α-tetrahydroprogesterone (ALLO), a molecule acting as a positive allosteric modulator of GABA-benzodiazepine (BZD) complex. This mechanism has been related with the antidepressant actions of FLX in humans and rodents (Khisti and Chodpe, 2000; Romeo et al., 1998). In addition, castration modifies the density of GABA-BZD receptors (Canonaco et al., 1993) without reducing the ALLO synthesis induced by FLX (Serra et al., 2001). Interestingly the acute administration of estradiol (10 μg/rat, s.c.) re-established the density of GABA-BZD complex in brain (Canonaco et al., 1993), thus this via could also participate in the synergism between FLX and E_2 here reported. On the other hand, the brain dopaminergic system participates in the effect of DMI in the FST (Cervo and Samanin, 1987). A reduction in dopamine brain levels together with an increase of its metabolism have

been found in Orx rats, but these effects are reversed by a chronic treatment with T (Putnam et al., 2001) or estradiol (0.5 μg/kg, s.c) (Bitar et al., 1991).

Changes in locomotor activity by pharmacological treatments can modify the results of the FST (Wieland and Lucki, 1990). It is possible that a reduction in immobility produced by the treatments, such as DMI (5 mg/kg) and FLX (10 mg/kg, respectively), E_2 (10–20 μg/rat), or the combinations of E_2 plus the antidepressant drugs, is due to unspecific effects as an increase in the ambulatory activity. However these treatments produced no changes on the ambulation or a reduction of the locomotor activity, arguing against this idea.

Although our results permit to establish a relationship between estradiol levels (in plasma, and probably in brain) and the effect of DMI and FLX in male rats, other possibilities should be explored to understand the role of gonadal hormones in the antidepressants' effects. Thus, it is interesting to evaluate the effect of antidepressant drugs in Orx rats pre-treated with a combination of T and E_2, both attaining physiological levels. Other issue that requires to be studied is the quantification of T and E_2 levels in brain after the treatments that modified the effect of antidepressant drugs. On the other hand, in clinical practice a long-term treatment with antidepressant drugs, and not a sub-chronic, reduces depression; thus, other studies using a chronic schedule with DMI and FLX in male rats should be conducted to establish a more precise comparison.

The present data indicate that estrogens play an important role in the behavioral effects of antidepressants in the FST. Moreover, physiological and pharmacological doses of E_2 interact with ineffective doses of DMI and FLX, respectively, to produce antidepressant-like effects in castrated males. Thus, steroid hormones, particularly E_2, participate in the effect of antidepressants in this animal model of depression. These results are in line with clinical reports indicating a decisive role of male gonadal steroids in affective disorders and in the effect of antidepressant drugs (Seidman and Rabkin, 1998).

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