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# Olanzapine plus 17-β estradiol produce antidepressant-like actions in rats forced to swim

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ABSTRACT

Several combinations of effective treatments have been used in the search for higher response rates or more rapid responses than monotherapy to diminish treatment-resistant depression. One strategy is to combine olanzapine plus antidepressant drugs. In preclinical studies in male rats, olanzapine combined with fluoxetine produce antidepressant-like actions and increase the allopregnanolone levels in the brain. 17-β estradiol also produces antidepressant-like actions by increasing allopregnanolone levels. However, the effects of combining olanzapine with 17-β estradiol in the forced swimming test have not been tested before. Thus, systemic injections of vehicle plus olanzapine, or fluoxetine (20.0 mg/kg; 25.0 mg/kg) or 17-β estradiol (10.0 μg/rat; 20.0 μg/rat) reduced immobility by increasing active behaviors, which were cancelled by finasteride (finasteride was used to block the endogenous production of allopregnanolone by the brain) in ovariectomized rats forced to swim. Subthreshold doses of olanzapine (2.5 mg/kg) combined with subthreshold doses of 17-β estradiol (5.0 μg/rat) produced antidepressant-like actions, as did the combination subthreshold dose of olanzapine (2.5 mg/kg) plus the subthreshold dose of fluoxetine (15.0 mg/kg). Finasteride cancelled the antidepressant-like actions of the several combinations used. It is concluded that olanzapine alone or combined with fluoxetine or estradiol reduced immobility by increasing swimming. In conclusion, olanzapine produces antidepressant-like actions alone or in combination with estradiol. These antidepressant-like actions of this combination were cancelled by finasteride.


## 1. Introduction

Several combinations of effective treatments have been used in the search for higher response rates or more rapid responses than monotherapy to diminish treatment-resistant depression (Dodd et al., 2005). One strategy is to combine olanzapine plus antidepressant drugs (Nemeroff, 2007). Accordingly, olanzapine plus fluoxetine is an approved therapy for treating human depression (Davis et al., 2005). In preclinical studies in male rats, olanzapine (Dubrovsky, 2005) or fluoxetine (Uzunov et al., 1996), alone (Payne et al., 2008) or combined (Marx et al., 2006), produce antidepressant-like actions and increase the allopregnanolone levels in the brain.

Another possible combination used to treat human depression is the combination 17-β estradiol plus fluoxetine, which reduces depression scores in women (Westlund and Parry, 2003). In laboratory animals, this combination also produces antidepressant-like actions in ovariectomized Wistar rats (Estrada et al., 2006). However, the combination olanzapine plus estradiol had not been tested before in ovariectomized rats. Therefore, we tested the possible antidepressant-like actions of olanzapine combined with systemic injections of 17-β estradiol in the forced swimming test (FST). In the clinic, all antidepressant drugs require about two or three weeks to produce antidepressant actions. However, in the present study, we followed the protocol developed by Porsolt et al. (1977) and modified by Detke and Lucki (1996). In this protocol, the antidepressants are administered in pharmacological doses at 23, 5 and 1 h prior to the test. The rats are forced to swim in a 15-min session and 24 h later they are re-tested in the pool again and the drug effects are evaluated.

We tested the hypothesis that olanzapine will combine with 17-β estradiol and that they will produce antidepressant-like actions in rats forced to swim; we also tested the idea that the combination olanzapine plus 17-β estradiol would probably produce antidepressant-like actions via the production of allopregnanolone. The conversion of progesterone to allopregnanolone in the brain by means of 5-α-reductase isoenzymes was blocked by the administration of finasteride (Kokate et al., 1999). There are gender differences in both the features of human depression and the effectiveness of antidepressants (Sloan

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and Kornstein, 2003). However, we used ovariectomized rats to avoid the gonadal steroids produced during the estrous cycle and we used finasteride to prevent the production of neurosteroids by the brain.

Considering that olanzapine may produce sedative actions in male rats (Ahnaou et al., 2003), we tested the rats in the open field test before applying the swimming test, in order to make sure that decreased immobility or increased active behaviors in the FST were not secondary to non-specific effects on locomotor activity due to the treatments.

## 2. Methods

### 2.1. Animals

Ovariectomized Wistar rats (250–300 g; $n=224$) were used. They were lodged in groups of five in housing facilities (room temperature: 20–22 °C; 12 light/12 dark cycle; lights on at 06:00 a.m.). The rats had free access to food (Harlan, Madison, Wisconsin, U.S.A.) and tap water. All experiments followed strict principles of animal care (National Institutes of Health, 1996).

### 2.2. Drugs

17-β estradiol (in 0.2 ml sesame oil plus some drops of dichloromethane; Sigma, St. Louis, MO, USA; s.c.), olanzapine (Eli Lilly Co.; Indianapolis, IN, USA; i.p.), fluoxetine (Lilly Research Laboratories; dissolved in 0.9% sodium chloride; s.c.) and the inhibitor of the enzyme 5α-reductase, finasteride (Steraloids, Newport, RI; dissolved in a 20% solution of 2-hydroxypropyl-b-cyclo-dextrin s.c.), were administered.

### 2.3. Experimental design

#### 2.3.1. Dose-dependent curves for drugs

Initially, dose-dependent curves for olanzapine (Dose 1 = 2.5; Dose 2 = 5.0; Dose 3 = 10.0 mg/kg), fluoxetine (Dose 1 = 15.0, Dose 2 = 20.0, and Dose 3 = 25.0 mg/kg) or 17-β estradiol (Dose 1 = 5.0; Dose 2 = 10.0; Dose 3 = 20.0 μg/rat) were obtained. Olanzapine, fluoxetine or 17-β estradiol was administered plus vehicle or plus finasteride (50 mg/kg; s.c.). Each experimental group included seven rats.

#### 2.3.2. Interaction experiments

During interaction experiments, subthreshold doses of several drugs were administered to different groups of rats: 1) the vehicle + vehicle + vehicle group; 2) the vehicle + vehicle + finasteride group; 3) the olanzapine (2.5 mg/kg) plus 17-β estradiol (5.0 μg/rat) + vehicle group; 4) the olanzapine (2.5 mg/kg) + fluoxetine (15.0 mg/kg) + vehicle group; 5) the fluoxetine (15.0 mg/kg) + 17-β estradiol (5.0 μg/rat) + vehicle group; 6) the olanzapine (2.5 mg/kg) plus 17-β estradiol (5.0 μg/rat) + finasteride group; 7) the olanzapine (2.5 mg/kg) + fluoxetine (15.0 mg/kg) + finasteride group; 8) the fluoxetine (15.0 mg/kg) + 17-β estradiol (5.0 μg/rat) + finasteride group.

All rats were tested first in the open field test and then in the FST. In accordance with Detke and Lucki (1996), two swim sessions were conducted: a 15-min pre-test session followed 24 h later by a 5-min test session. The test session was done just after the open field test. Systemic injections of olanzapine or fluoxetine were administered three times to the correspondent experimental groups at the intervals: 23, 5 and 1 h prior to the test. 17-β estradiol was administered 48 h before the FST. Finasteride was administered at a 50 mg/kg dose, 4 h and 1.5 h before the experimental sessions. The dose of finasteride and the time interval of injection were obtained after a pilot study and following a study by Lephart et al. (1996). The time interval between injections and observations were chosen accordingly to Detke and Lucki (1996) and Molina et al. (2008) for olanzapine and fluoxetine; and for 17-β estradiol, the time interval was followed according to Walf and Frye (2005), Martínez et al., (2008) and after a pilot study. After that, behavioral tests were conducted.

### 2.4. Behavioral tests

#### 2.4.1. Open field test

The rats were placed individually in the middle of an open field apparatus (Plexiglas cage: height: 20.0 cm; length: 44.0 cm; width: 33.0 cm). Twelve squares (11 × 11 cm) were delineated on the floor. The squares crossed were counted by an observer blind to the experimental groups, over a 5-min period. After each trial, the open field apparatus was cleaned. The open field test was video-taped and an observer unaware of the treatments scored the test. Many scientific authors that use the FST also use the open field test to reveal non-specific effects of drug treatments. Some authors performed the open field test before the FST (Gavioli et al., 2003; Gutiérrez et al., 2007); others performed it after the FST (Brotto et al., 2000; Harkin et al., 1999). We preferred to perform the open field test first, since it cannot affect the results coming from the FST, which is stressful (Borsini and Meli, 1988).

#### 2.4.2. Forced swimming test

The rats were placed individually in a glass cylinder (46 cm tall × 20 cm in diameter; containing water: 23–25 °C). Since rats in deeper water exhibit more swimming and climbing behaviors, and differences in behaviors produced by specific kinds of antidepressants are magnified when the rats are tested in deeper water (Detke and Lucki, 1996), the depth of 30.0 cm was selected. The water was deep enough to prevent the rats from touching the bottom of the aquarium. The habituation session consisted in placing the rats in the aquarium during 15 min. Twenty-four hours later, in a 5-min test session; the rats were placed in the aquarium again. At the end of each 5-s period during the test session, passive immobility, i.e., when the rats remained floating in the water, making only the necessary movements to keep their heads above water (Cervo et al., 1988); and the active behaviors: swimming, i.e., when the rats made active swimming motions; and climbing, i.e., when they made vigorous movements with their forepaws in and out of the water, usually directed against the walls (Detke and Lucki, 1996) were scored. All swimming sessions were video-taped and scored by an observer unaware of the experimental groups. After swimming, the rats were placed in a dry chamber (30.0 ± 1.0 °C).

### 2.5. Ovariectomy

Before undergoing drug and behavioral procedures, the rats were ovariectomized. They were anesthetized with ether anesthesia and two lateral incisions were made to expose and remove the ovaries. After suturing their muscles and skin, we allowed the animals to recover during two weeks. After recuperation, the drug experiments and behavioral tests began.

### 2.6. Statistical analysis

The data (mean ± SEM) coming from the dose-dependent study were analyzed by means of a two-way analysis of variance (ANOVA) with each drug (vehicle plus three doses) serving as one factor and finasteride versus saline serving as a second factor. Where significant effects were found, the Tukey test was used as a post-hoc test. Differences were considered statistically significant only if $P \leq 0.05$.

Data obtained in the interaction drugs were analyzed by means of the two-way ANOVA. The main factors are: A) fluoxetine/olanzapine/estradiol versus vehicle and B) finasteride versus vehicle. Where significant effects were found, the Tukey test was used as a post-hoc

test. Differences were considered statistically significant only if $P \leq 0.05$.

## 3. Results

### 3.1. Dose-dependent study

#### 3.1.1. Forced swimming test

Olanzapine reduced immobility (two-way ANOVA; $F$ (1,3) = 128.66, $P<0.001$; 5.0 mg/kg, $P<0.05$; 10.0 mg/kg, $P<0.05$) (Fig. 1) by increasing swimming (ANOVA; $F$ (1,3) = 102.35; $P<0.001$) (Fig. 2). Finasteride canceled the effects of olanzapine (Figs. 1 and 2).

Fluoxetine reduced immobility (two-way ANOVA; $F$ (1,3) = 87.9, $P<0.001$; 20.0 mg/kg, $P<0.05$; 25.0 mg/kg, $P<0.05$) (Fig. 3) by increasing swimming (ANOVA; $F$ (1,3) = 114.5; $P<0.001$) (Fig. 4). Finasteride canceled the effects of fluoxetine (Figs. 3 and 4).

17-β estradiol reduced immobility (two-way ANOVA; $F$ (1,3) = 105.65, $P<0.001$; 10.0 μg/rat, $P<0.05$; 20.0 μg/rat, $P<0.05$) (Fig. 5) by increasing swimming (ANOVA; $F$ (1,3) = 125.6; $P<0.001$) (Fig. 6). Finasteride canceled the effects of 17-β estradiol in the FST (Figs. 5 and 6). Olanzapine or fluoxetine or estradiol did not modify climbing behavior.

#### 3.1.2. Ambulatory behavior

Olanzapine (two-way ANOVA; $F$ (1,3) = 97.58, $P<0.001$; 5.0 mg/kg, $P<0.05$; 10.0 mg/kg, $P<0.05$) or fluoxetine (two-way ANOVA; $F$ (1,3) = 123.25, $P<0.001$; 20.0 mg/kg, $P<0.05$; 25.0 mg/kg, $P<0.05$) or 17-β estradiol (two-way ANOVA; $F$ (1,3) = 84.5, $P<0.001$; 10.0 μg/rat, $P<0.05$; 20.0 μg/rat, $P<0.05$) reduced locomotion in the open field test. Finasteride canceled the effects of olanzapine, fluoxetine or 17-β estradiol in the open field test (Table 1).

### 3.2. Interaction study

#### 3.2.1. Forced swimming test

During the interaction study, several combinations of drugs reduced immobility behavior (two-way ANOVA test; $F$ (1,3) = 120.36; $P<0.001$). Accordingly, vehicle combined with: A) subthreshold dose of olanzapine and subthreshold dose of estradiol ($P<0.05$); B) the subthreshold dose of olanzapine plus the subthreshold dose of fluoxetine ($P<0.05$); or C) the subthreshold dose of 17-β estradiol plus subthreshold dose of fluoxetine ($P<0.05$) reduced immobility by elevating active behaviors.

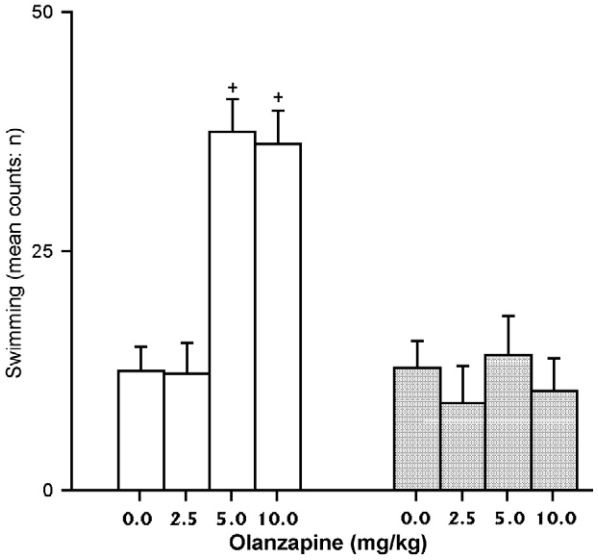


**Fig. 2.** Olanzapine injected plus the vehicle (open bars) in which finasteride was dissolved increased swimming behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of olanzapine in swimming behavior. +$P<0.05$ against the respective vehicle group.

Swimming behavior was the active behavior that was increased (two-way ANOVA test; $F$ (1,3) = 135.24; $P<0.001$) by the combinations: vehicle plus olanzapine plus estradiol ($P<0.05$); vehicle plus olanzapine plus fluoxetine ($P<0.05$); or by the combination vehicle plus estradiol plus fluoxetine ($P<0.05$). These effects of drugs were cancelled by finasteride (Table 2).

#### 3.2.2. Ambulatory test

In the open field test (two-way ANOVA test; $F$ (1, 3) = 216.28; $P<0.001$), vehicle combined with olanzapine plus fluoxetine ($P<0.05$), olanzapine plus estradiol ($P<0.05$) or estradiol plus fluoxetine ($P<0.05$) reduced locomotion. These effects were canceled by finasteride (Table 3).

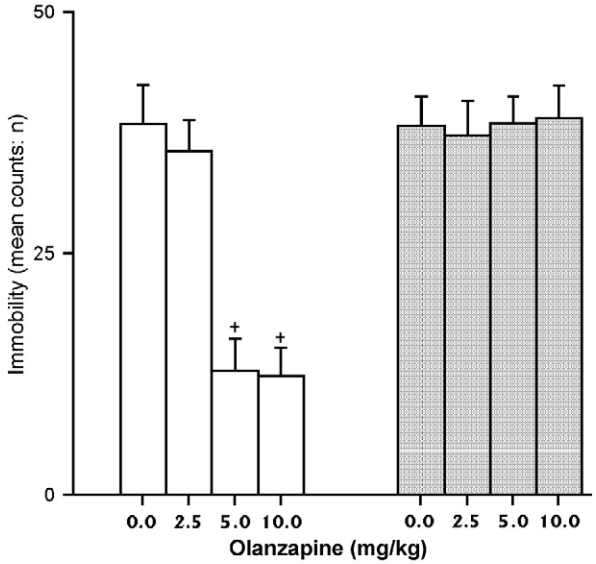


**Fig. 1.** Olanzapine injected plus the vehicle (open bars) in which finasteride was dissolved reduced immobility behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of olanzapine in immobility. +$P<0.05$ against the respective vehicle group.

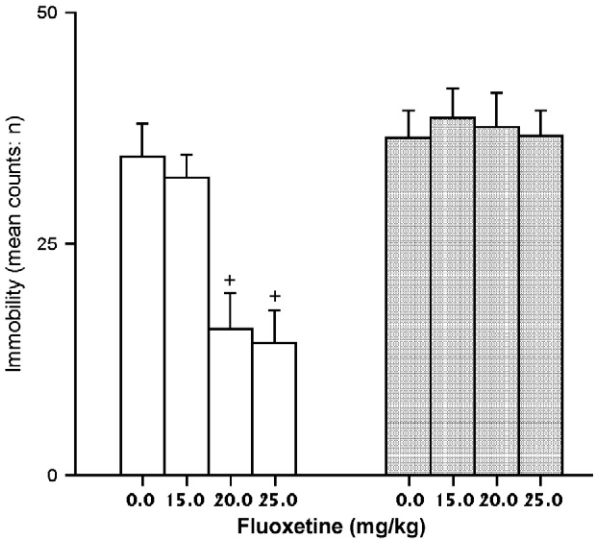


**Fig. 3.** Fluoxetine injected plus the vehicle (open bars) in which finasteride was dissolved reduced immobility behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of fluoxetine in immobility. +$P<0.05$ against the respective vehicle group.

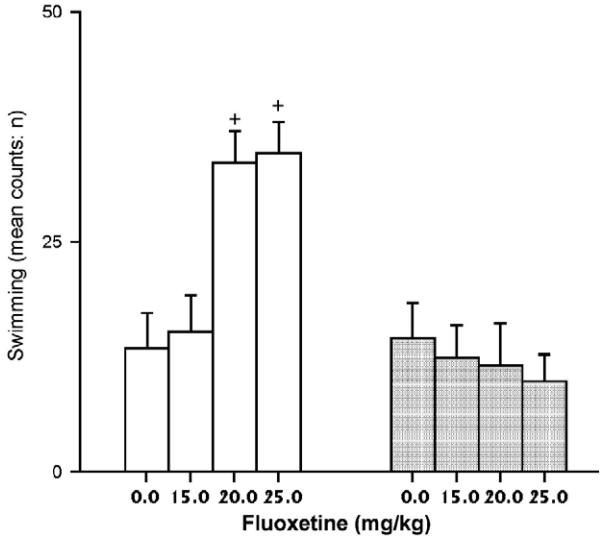


**Fig. 4.** Fluoxetine injected plus the vehicle (open bars) in which finasteride was dissolved increased swimming behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of fluoxetine in swimming behavior. $+P<0.05$ against the respective vehicle group.

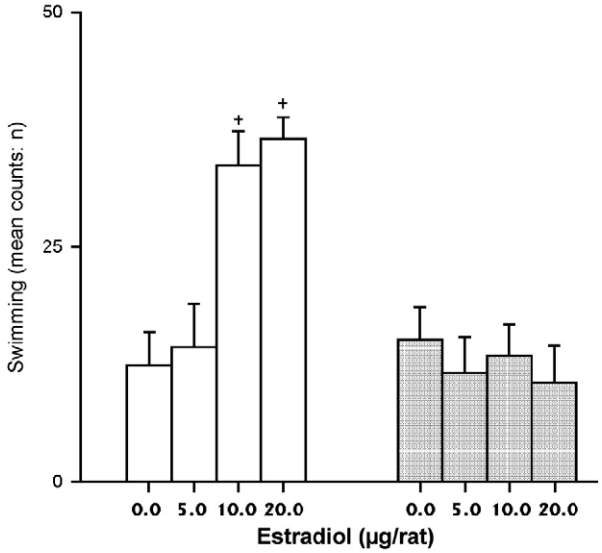


**Fig. 6.** 17-β estradiol injected plus the vehicle (open bars) in which finasteride was dissolved increased swimming behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of 17-β estradiol in swimming behavior. $+P<0.05$ against the respective vehicle group.

## 4. Discussion

This study verified the hypothesis that olanzapine plus 17-β estradiol will produce antidepressant-like actions in ovariectomized Wistar rats tested in the FST. The results showed that: 1) olanzapine or fluoxetine or 17-β estradiol administered plus vehicle reduced immobility by increasing swimming, these effects being cancelled by finasteride; 2) during combination experiments, subthreshold doses of olanzapine plus subthreshold doses of fluoxetine or 17-β estradiol reduced immobility by increasing swimming, as did subthreshold doses of fluoxetine plus subthreshold doses of 17-β estradiol; 3) finasteride cancelled the antidepressant-like actions produced by these different combinations of drugs.

The FST is a test designed to evaluate the antidepressant activity of drugs (Porsolt et al., 1977); it represents an aversive stressful situation from which the rats cannot escape, thus generating behavioral despair, i.e., immobility (Borsini and Meli, 1988). Most of the published papers testing antidepressant drugs in animals use the forced swimming test with water only 15 to 17 cm deep and evaluate only the amount of immobility behavior displayed by the rats. In this study, we have followed the technique designed by Detke et al. (1995). In this technique not only immobility is evaluated, but also other active behaviors such as swimming and climbing are registered. This modification to the FST provides a more refined analysis of a purely behavioral assay and is used to distinguish drugs having a common therapeutic outcome, but acting on distinct neurotransmitter systems. Accordingly, antidepressant drugs with predominant noradrenergic- or dopaminergic-elevating effects reduce immobility by increasing climbing behavior. Conversely, antidepressant drugs with predominant serotonin-elevating effects reduce immobility by increasing swimming behavior (Detke and Lucki, 1996). The data obtained for olanzapine, fluoxetine (Molina et al., 2008) and 17-β estradiol (Martínez et al., 2008) are in line with the aforementioned findings.

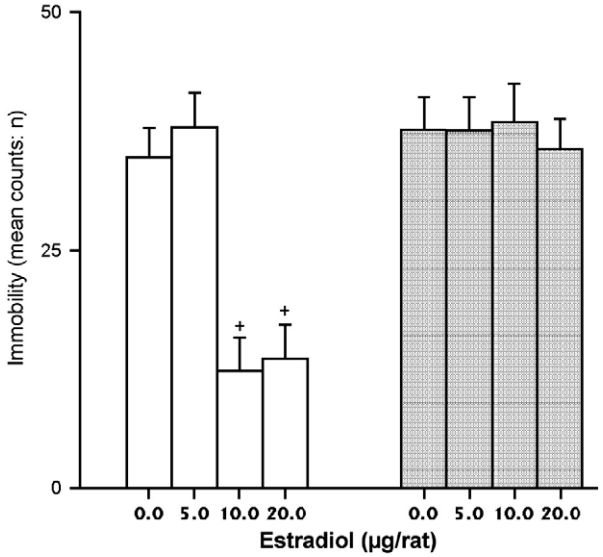


**Fig. 5.** 17-β estradiol injected plus the vehicle (open bars) in which finasteride was dissolved reduced immobility behavior in the forced swimming test. Finasteride (dashed bars) cancelled the effects of 17-β estradiol in immobility. $+P<0.05$ against the respective vehicle group.

In this study, our rats were exposed to the water twice, first in a pre-test session and then in the test session. The evaluation of drug effects was done only during the test. This paradigm may include drug effects on learning or memory. In the forced swimming test during the pre-test session, the rats are forced to swim in a confined space from which they cannot escape (Korte, 2001). In this condition, the rats stop active behaviors and start the behavior of immobility. On the second day, the rats are tested again in the pool and the rats show the same behavior, i.e., immobility (Korte, 2001). The Porsolt test has been used widely to test the antidepressant effects of drugs. Immobility has been interpreted as a learned behavior of despair and has been considered as an animal model of human depression; other authors consider that the rats learn to be immobile. However, all the antidepressant drugs tested in this paradigm reduce immobility 24 h after the treatments, in contrast to the weeks required for recovery from clinical depression (Korte, 2001).

Olanzapine alone or combined with fluoxetine reduced immobility by increasing swimming behavior. Augmenting antidepressant drugs with atypical antipsychotics for treatment-resistant major depression is a pharmacological option in treating human depression (Shelton and Papakostas, 2008), and olanzapine is an option (Seddon and Nutt, 2007). In the clinic, the efficacy of the combination olanzapine plus fluoxetine (Vieta, 2005) in producing antidepressant actions can be explained by modifications in the serotonergic pathway (Klint and

**Table 1**
Effects of vehicle plus systemic injections of antidepressant treatments (dose response curves) in locomotion (number of squares crossed) of rats tested in the open field test (mean ± S.E.M.).

| | Vehicle | | | Finasteride | | |
|---|---|---|---|---|---|---|
| | Olanzapine | Fluoxetine | Estradiol | Olanzapine | Fluoxetine | Estradiol |
| Vehicle | 65.4 ± 12.5 | 67.2 ± 11.7 | 66.2 ± 15.4 | 66.7 ± 12.5 | 68.8 ± 17.5 | 62.8 ± 13.4 |
| Dose 1 | 63.3 ± 12.7 | 64.3 ± 13.6 | 62.3 ± 14.2 | 65.8 ± 13.4 | 60.4 ± 11.4 | 63.9 ± 12.4 |
| Dose 2 | 32.4 ± 13.6* | 31.3 ± 14.1* | 33.2 ± 8.9* | 66.8 ± 12.6 | 60.2 ± 10.8 | 65.9 ± 10.6 |
| Dose 3 | 30.6 ± 12.9* | 28.9 ± 13.0* | 30.2 ± 10.8* | 67.5 ± 11.9 | 61.3 ± 12.4 | 62.5 ± 13.3 |

Olanzapine (Dose 1 = 2.5; Dose 2 = 5.0; Dose 3 = 10.0 mg/kg), fluoxetine (Dose 1 = 15.0, Dose 2 = 20.0, and Dose 3 = 25.0 mg/kg), and 17-β estradiol (Dose 1 = 5.0; Dose 2 = 10.0; Dose 3 = 20.0 μg/rat) were administered intraperitoneally.
* $P<0.05$ against the respective vehicle group.

Weikop, 2004). The aforementioned findings may explain the antidepressant-like actions produced by olanzapine plus fluoxetine (an antidepressant drug that impinges on the serotonergic pathway). However, since olanzapine produces some undesirable side effects in human beings (Finkel, 2004) we decided to test another combination, i.e., olanzapine plus estradiol.

Estradiol or subthreshold doses of estrogens alone or combined, either with olanzapine or fluoxetine, produced antidepressant-like actions. The antidepressant-like actions of estrogens have been demonstrated in ovariectomized rats (Walf and Frye, 2008). These antidepressant-like actions of estrogens are probably mediated via stimulation of 5HT-1A receptors in ovariectomized female Wistar rats (Estrada et al., 2006). An interesting scientific finding is that the pharmacological treatments used to diminish depression increase allopregnanolone levels (Eser et al., 2006b). Thus, we studied whether finasteride impinges on the antidepressant-like actions of olanzapine, fluoxetine or estradiol.

The antidepressant effects of olanzapine, fluoxetine, estradiol or the combination fluoxetine plus olanzapine or estradiol plus fluoxetine were canceled by finasteride. Finasteride has been used to cancel the production of allopregnanolone by brain cells and it has been demonstrated that allopregnanolone may play a vital role in human depression (Uzunova et al., 2004). Treatments with selective serotonin reuptake inhibitors restore allopregnanolone levels and ameliorate depression (Pinna et al., 2006). In ovariectomized rats, allopregnanolone produces antidepressant-like actions (Frye and Walf, 2002; Molina-Hernández et al., 2005) and active stressors increase allopregnanolone levels in the brain (Frisone et al., 2002); this suggests that changes in brain neurosteroids may be related to the effects of several antidepressant treatments (Pinna et al., 2006). In this study, we also found that finasteride cancelled those antidepressant-like actions of estradiol, which suggests that estrogens may produce antidepressant-like actions probably due to an elevation in allopregnanolone levels. We have not found experimental evidence to support this finding regarding depression, but Frye and Rhodes, in ovariectomized rats, found that estrogen-priming can enhance progesterone's anti-seizure effects in part by increasing the hippocampal levels of allopregnanolone (Frye and Rhodes, 2005), so these findings might explain our results. However, it has been suggested that the changes in allopregnanolone levels after antidepressant treatments may reflect direct pharmacological effects of the antidepressants used rather than clinical improvement in general (Eser et al., 2006a), because non-pharmacological antidepressant treatment strategies did not affect allopregnanolone levels (Eser et al., 2006b).

A possible criticism of our study is that we only utilized ovariectomized rats to evaluate the antidepressant-like actions of drugs. This criticism arises because it has been demonstrated by Okada et al. (1997) that bilateral ovariectomy prolongs the immobility levels in the FST. This prolongation of immobility time is longer than the time in immobility showed by intact rats and sham rats. Besides, ovariectomy is a surgical menopause that produces dramatic influences on the neurotransmitter systems, membrane stability and susceptibility to depression (Seeman and Ross, 2007). We used ovariectomized rats to avoid the gonadal steroids produced during the estrous cycle and we used finasteride to prevent the production of neurosteroids by the brain. An interesting idea is to repeat our experiment in intact rats during the estrous cycle, considering that neuronal firing (Contreras et al., 2000) and the effects of several drugs (Molina et al., 2002) are related to the estrous cycle phases. Further experiments should be performed by testing intact animals during several estrous phases.

### 4.1. Effects of drugs in the open field test

In the open field test, olanzapine or fluoxetine or 17-β estradiol reduced locomotion. Some authors claim that the open field test does not involve aversive stimulation (Crawley, 1985). However, the open field test can be used as an animal model of anxiety (Treit and Fundytus, 1988). Many researchers use it as a complement to the FST to avoid false positive results that can be obtained in the FST, since

**Table 2**
Effects of subthreshold injections of olanzapine (2.5 mg/kg) plus subthreshold doses of fluoxetine (15.0 mg/kg) or estradiol (5.0 μg/rat) combined with finasteride or vehicle in rats forced to swim (mean ± S.E.M.).

| Treatments | Immobility | Swimming | Climbing |
|---|---|---|---|
| Vehicle + vehicle + vehicle | 38.4 ± 12.4 | 11.3 ± 2.5 | 10.3 ± 2.8 |
| Olanzapine + estradiol + vehicle | 12.4 ± 3.2* | 39.8 ± 13.4* | 7.8 ± 2.0 |
| Olanzapine + fluoxetine + vehicle | 11.9 ± 3.3* | 38.0 ± 12.4* | 10.1 ± 2.1 |
| Estradiol + fluoxetine + vehicle | 12.8 ± 2.8* | 35.6 ± 11.7* | 11.6 ± 2.4 |
| Vehicle + vehicle + finasteride | 37.9 ± 11.7 | 10.8 ± 3.2 | 11.3 ± 3.0 |
| Olanzapine + estradiol + finasteride | 35.6 ± 10.8 | 10.8 ± 3.8 | 13.6 ± 3.7 |
| Olanzapine + fluoxetine + finasteride | 37.8 ± 12.3 | 11.2 ± 3.2 | 11.0 ± 3.6 |
| Estradiol + fluoxetine + finasteride | 36.5 ± 11.0 | 9.8 ± 2.8 | 13.7 ± 3.0 |

At the end of each 5-s period, the rat's behavior at that time was scored, i.e., immobility, swimming or climbing (mean counts).
* $P<0.05$ against the vehicle + vehicle + vehicle group.

**Table 3**
Effects of subthreshold injections of olanzapine (2.5 mg/kg) plus subthreshold doses of fluoxetine (15.0 mg/kg) or estradiol (5.0 μg/rat) combined with finasteride or vehicle in rats tested in the open field test (mean ± S.E.M.).

| Treatments | Locomotion |
|---|---|
| Vehicle + vehicle + vehicle | 68.8 ± 12.5 |
| Olanzapine + estradiol + vehicle | 30.2 ± 10.2* |
| Olanzapine + fluoxetine + vehicle | 32.4 ± 5.8* |
| Estradiol + fluoxetine + vehicle | 33.3 ± 11.7* |
| Vehicle + vehicle + finasteride | 70.2 ± 13.5 |
| Olanzapine + estradiol + finasteride | 66.5 ± 10.9 |
| Olanzapine + fluoxetine + finasteride | 67.5 ± 13.9 |
| Estradiol + fluoxetine + finasteride | 69.2 ± 13.4 |

* $P<0.05$ against the vehicle + vehicle + vehicle group.

some antidepressant drugs may also produce sedative effects (Delini-Stula et al., 1995), as olanzapine, fluoxetine or 17-β estradiol did. In the present study, we performed the open field test before the forced swimming test, so a possible criticism of our data is that stress from the open field test might affect the results of the FST. Other authors perform the open field after the FST, and they are concerned that the stress produced by the swimming sessions may affect the open field test. Perhaps the drug effects would have been different if the open field had not been conducted immediately before the FST. This criticism can be answered by comparing the different groups against the control group, as this group received only vehicle and displayed certain levels of immobility in the FST. Conversely, those rats that received antidepressant doses of olanzapine, fluoxetine or 17-β estradiol displayed reduced open field activity but increased their active behaviors in the FST. The fact that olanzapine, fluoxetine, 17-β estradiol or the different combinations used did not induce hyperlocomotion, but in some cases decreased motor activity in the open field test, leads us to the conclusion that the effects of these drugs in the FST cannot be attributed to an increase in motor activity but can be considered as antidepressant-like actions.

In conclusion, olanzapine produces antidepressant-like actions alone or in combination with estradiol. This suggests that the combination olanzapine–estradiol could be a therapeutic option for treating human depression. Further experiments should be performed before reaching final conclusions.

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