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Acute treatment with 5-HT3 receptor antagonist, tropisetron, reduces immobility in intact female rats exposed to the forced swim test

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Abstract

The effects of tropisetron, a 5-HT3 receptor antagonist, were evaluated in adult Fischer female rats exposed to the Forced Swim Test (FST). Rats selected on the days of proestrus or estrus was immersed in a cylinder of water for 2 consecutive days. Rats were exposed to the FST for 15 min on day 1 (pretest), followed by a 5-min session (test), 24 h later. The proestrous–estrous group consisted of rats that were exposed to the FST on their proestrous stage (pretest); then 24 h later the same rats were exposed to the FST on their estrous stage (test). Rats in the estrous–diestrous group were exposed to the FST on their estrous stage (pretest) and 24 h later on their diestrous stage (test). Rats were injected intraperitoneally with saline or 1.0 or 2.0 mg/kg tropisetron 30 min prior to exposure to the cylinder on the test day. Immobility, swimming, and struggling behaviors were scored for 5 min. There was a significant decline in immobility after treatment with 2.0 mg/kg tropisetron in both groups. In addition, a significant decline in swimming was observed in the estrous rats (proestrous–estrous group) after treatment with 2.0 mg/kg tropisetron. There were no significant effects of tropisetron on struggling in any groups examined.

Keywords: Tropisetron; Female rats; Immobility; Forced Swim Test

1. Introduction

Female gonadal hormones such as estrogen and progesterone modulate the functioning of neurotransmitters such as serotonin (5-HT) (Bethea et al., 2002; Bhatnagar et al., 2004; Biegon and Bercovittz, 1980; Farmer et al., 1996; Frankfurt et al., 1994; Gundlah et al., 1998; Li et al., 2004; Maswood et al., 1999; Uphouse, 2000; Wetzel et al., 1998). Hormonal modulation of 5-HT by these gonadal steroids has important implications since several physiological as well as pathophysiological functions are regulated by this neurotransmitter. Serotonin's regulation of these functions is mediated by its binding to multiple receptors located both in the central and in the peripheral nervous system (for review see Hannon and Hoyer, 2002). With the exception of, the 5-HT3 receptors, which belong to the ligand-gated ion channel receptor superfamily, the majority of 5-HT receptors are G-protein coupled receptors (Hannon and Hoyer, 2002; Lummis, 2004; Maricq et al., 1991). Activation of the 5-HT3 receptors causes cation channels to open leading to excitatory responses (Hannon and Hoyer, 2002; Maricq et al., 1991; Sugita et al., 1992). The 5-HT3 receptors are involved in modulating several functions such as anxiety/depression, emesis, pain, addiction and irritable bowl syndrome (Costall and Naylor, 2004; Dukat, 2004; Martin et al., 1992; Wolf, 2000). In particular, treatment with 5-HT3 receptor antagonists reduces behavioral indices of both anxiety and depression (Bourin et al., 2004; Costall and Naylor, 2004; Dukat, 2004; Greenshaw, 1993; Nakagawa et al., 1998; Wolf, 2000).

Anxiety and depression are more prevalent in females than in males (Caldecott-Hazard et al., 1999; Kendler, 1998; Kendler et al., 2000; Kessler et al., 1994; Stahl, 1998; Steiner et al., 2003). Although the precise mechanism of females enhanced vulnerability to anxiety/depression are not known, fluctuations in gonadal hormones are implicated as contributing factors for such gender disparity (Fink et al., 1996; Caldecott-Hazard et al., 1999; Kessler et al., 1994; Richardson and Robinson, 2000; Robinson, 2001; Steiner et al., 2003). In both basic and clinical studies, treatment with estrogen reduces indices of depressive behaviors in females (Estrada-Camarena et al., 2003; Rachman et al., 1998; Robinson,

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2001). Furthermore, the actions of some antidepressants can be accentuated after estrogen is added to the treatment regime (Estrada-Camarena et al., 2004; Richardson and Robinson, 2000; Stahl, 1998). Hormones such as estrogen and progesterone modulate the functioning of several 5-HT receptors, including the 5-HT3 receptors, which are linked to anxiety and depression (Fink et al., 1996; Li et al., 2004; Oz et al., 2002; Uphouse, 2000; Wetzel et al., 1998). More specifically, estrogen and progesterone are thought to act as antagonists at 5-HT3 receptors (Oz et al., 2002; Wetzel et al., 1998). Furthermore, treatment with estrogen together with progesterone decreases the expression of 5-HT3 mRNA in rats exposed to stress (Li et al., 2004).

If blocking the actions of 5-HT3 receptors attenuates anxiety/depression (Greenshaw, 1993; Martin et al., 1992; Nakagawa et al., 1998; Redrobe and Bourin, 1997; Wolf, 2000), then during the reproductive cycle when estrogen/progesterone levels are high, indices of anxiety/depression may be reduced through the action of these hormones on the 5-HT3 receptors. Such specific interactions between 5-HT3 receptors and gonadal steroids may provide further mechanism to explain female's enhanced vulnerability to mood disorders.

The forced swim test (FST) is a behavioral model of despair that is used to screen the effectiveness of mood enhancing compounds in rodents (Cryan et al., 2002; Lucki, 1997; Porsolt et al., 1977). In this model, when a rat or mouse is exposed to acute stress by being immersed in an inescapable cylinder full of water, the animal exhibits an immobile behavior. Immobility is displayed when the rat stops struggling and makes just enough movement to keep floating (Cryan et al., 2002; Lucki, 1997; Porsolt et al., 1977). Consequently, compounds that decrease immobility and/or increase active behaviors such as struggling/swimming in the FST are generally thought to be able to reduce indices of depression (Cryan et al., 2002; Lucki, 1997; Porsolt et al., 1977). Although the FST is mainly used to screen the efficacy of mood enhancing compounds, it is also used as an effective stressor, because exposure to the FST itself causes a variety of neurobiological changes that are typical of other stressors (Connor et al., 1997; Drossopoulou et al., 2004; Korz and Frey, 2003).

Various behavioral and neurochemical changes associated with the FST have been evaluated predominantly in male rodents (Cryan et al., 2002; Lucki, 1997; Porsolt et al., 1977; Redrobe and Bourin, 1997). However, when female rats were included in the FST, gender as well as estrous cycle differences in behavioral, neurochemical and endocrine parameters were reported (Alonso et al., 1991; Barros and Ferigolo, 1998; Bellindo et al., 2003; Bhatnagar et al., 2004; Contreras et al., 1998; Drossopoulou et al., 2004; Jones and Lucki, 2005; Marvan et al., 1996, 1997). Male rats exhibit greater immobility than females (Alonso et al., 1991; Barros and Ferigolo, 1998; Bhatnagar et al., 2004); female rats tested during their diestrous stage (with low estrogen levels) display higher immobility than females in the proestrous stage (Contreras et al., 1998; Frye and Walf, 2002; Marvan et al., 1996, 1997). In the hypothalamus a decrease in 5-HT activity is observed in female rats, while males experience an increase in 5-HT activity after exposure to the FST (Drossopoulou et al., 2004). Hormone treatment in ovariectomized female rats also influence behavioral and neurochemical indices associated with the FST. For example, treatment of ovariectomized rats with estrogen decreases c-FOS expression as well as immobility behavior (Estrada-Camarena et al., 2003; Okada et al., 1997; Rachman et al., 1998). Estrogen-treatment also enhances the actions of antidepressants in rats by causing a decrease in immobility and an increase in swimming and climbing behavior (Estrada-Camarena et al., 2004). Similar to the antidepressant-like effects of estrogen in the FST, treatment with progesterone also reduces immobility behavior (Frye and Walf, 2002; Hernandez-Molina and Tellez-Alcantara, 2001; Martinez-Mota et al., 1999).

5-HT3 receptor antagonists decrease immobility duration of male rats (Nakagawa et al., 1998), and actions of certain antidepressants such as SSRIs include effects on the 5-HT3 receptors (Nakagawa et al., 1998; Redrobe and Bourin, 1997). The effects of 5-HT3 receptor antagonists have not been specifically evaluated in female rats exposed to the FST. However, 5-HT3 receptors may play a greater role in attenuating depressive behaviors in females than in male rats. For example, when behaviors such as immobility and swimming were evaluated in male and female wild type (WT) and 5-HT3 receptor knock-out (KO) mice, deletion of 5-HT3 receptors in females enhanced indices of depressive behavior (Bhatnagar et al., 2004). Modulation of 5-HT3 receptors by gonadal hormones may be a contributing factor for such differences (Oz et al., 2002; Wetzel et al., 1998). Consequently, if gonadal hormones such as estrogen, blocks the 5-HT3 receptors, then the response to 5-HT3 receptor specific drugs may be influenced by fluctuating levels of estrogen.

In the following study the FST was used to examine the effects of a 5-HT3 receptor antagonist on immobility, swimming and struggling behaviors in female rats during different stages of their reproductive cycle. Since estrogen and progesterone levels fluctuate across the rat's reproductive cycle, and since estrogen/progesterone are thought to inhibit the functioning of the 5-HT3 receptors, the response to tropisetron was expected to vary across the stages of the cycle.

2. Methods

2.1. Animals

Adult female Fischer (CDF-344) rats, 90–120 days old were purchased as adults or were bred at Whitely Psychology Laboratories from stock obtained from Harlan laboratory (Indianapolis, IND). Rats were housed in pairs in polycarbonate "shoebox" cages with food and water available ad lib. The housing rooms were maintained under a reversed light–dark cycle with lights off at 12:00 noon. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Franklin and Marshall College and conducted in compliance with the PHS policy for the care and use of animals.

2.2. Estrous cycle monitoring

Vaginal smears of intact females were monitored daily. Smears containing nucleated and/or cornified epithelial cells but

no leukocytes were considered proestrous smears. Estrous smears consisted mainly of cornified cells. Proestrous or estrous female rats that had been monitored for at least two complete estrous cycles were selected on the basis of their smears. Thirty-eight female rats were selected for use in the experiments based on their smear history and vaginal smears on the day of testing. There were two experimental groups, the proestrous–estrous group, and the estrous–diestrous group. Each group of rats was exposed to the FST twice. The proestrous–estrous group consisted of rats that were exposed to the FST first on their proestrous, or ovulatory stage (pretest); on day 2 (test), when treatment was initiated and behaviors recorded/reported, these rats were in their post-ovulatory or estrous stage. Levels of both estrogen and progesterone are elevated in the proestrous rats (Freeman, 1988). Levels of estrogen and progesterone start to decrease on the post-ovulatory or the estrous stage (Freeman, 1988). The second experimental group, the estrous–diestrous group consisted of a different set of rats. Rats in this group were first exposed to the pretest session during their estrous stage (post-ovulatory) on day one; on day two when treatment was initiated, these rats were in the stage of diestrus 1. During diestrus 1, estrogen levels are the lowest, but progesterone levels are higher than in the estrous rats (Freeman, 1988).

2.3. Drugs

Tropisetron (3-tropanylindole-3-carboxylate hydrochloride) was purchased from Sigma Aldrich. Tropisetron was dissolved in physiological saline (0.9% NaCl).

2.4. Forced Swim Test (FST)

All experiments were carried out between 13:00 and 17:00 in a dark room illuminated by red light bulbs to aid visibility. All sessions were recorded by a video camera. The FST consisted of a two-day testing procedure. During the pretest session, rats were individually immersed for 15 min into a Plexi-glass cylinder (95 cm high, 25 cm diameter) filled with 40 cm of water maintained at 25–27 °C. Twenty-four hours later, the same rats were exposed to a 5-min test session. Thirty-minutes before the test session, rats received intraperitoneal (IP) injections of either 0.9% saline; or 1.0 mg/kg or 2.0 mg/kg of tropisetron. In the proestrous–estrous group, $n=6$ estrous rats received saline treatment; $n=6$ received injections with 1.0 mg/kg tropisetron, and $n=6$ rats received injections with 2.0 mg/kg tropisetron. In the estrous–diestrous group, $n=7$ diestrous rats received saline injections; $n=7$, and $n=6$ diestrous rats were injected with 1.0 mg/kg and 2.0 mg/kg tropisetron respectively. Each rat was dried with a cotton towel after each swimming session. The immobility, swimming and struggling behaviors were scored every 5 s during the test session. An individual blind to the treatment conditions conducted all observations. Immobility was scored when the rat either stopped moving completely or made only the necessary movements to maintain the head above the water. Swimming was scored when the rat made coordinated and sustained movements with all four limbs, usually traveling around the interior of the cylinder without breaking the surface of the water with forelimbs. Struggling (or climbing) differed from swimming in that the front limbs were required to break the surface of the water. The active movements with the forepaws were mostly directed against the walls of the cylinder.

2.5. Open-field test

In order to rule out any effects of tropisetron on general locomotor activity, the effects of tropisetron were examined using Open-field Test (OFT). The open-field apparatus consisted of a circular arena constructed with ply-wood (96 cm diameter), with a 19-cm high aluminum wall. The floor of the apparatus was divided into a total of 28 segments. Rats were exposed to the FST for 15 min on day one. Twenty-four hours later, rats were injected with either saline or 2.0 mg/kg tropisetron. Thirty minutes after the injection individual rats were placed in the center of the open-field apparatus for 5 min. The total locomotor activity was measured by recording the total number of segments entered during the 5-min session. A rat was considered as entering a segment only when at least ¾ of the body had crossed the divided lines. The number of animals in each group are as follows saline estrous $n=9$; 2.0 mg/kg tropisetron estrous $n=9$; saline diestrous $n=9$; 2.0 mg/kg tropisetron diestrous $n=8$.

2.6. Statistical analysis

Data were organized as mean number of counts ± standard error (S.E.) during the 5-min test session for immobility, swimming and struggling behaviors in each condition. A 2 × 3 factor ANOVA was used to analyze the effects of tropisetron treatment on immobility or swimming/struggling behaviors of estrous and diestrous rats. Within each stage, a Tukey's test was used to compare treatment groups with the saline group or treatment groups with each other. For the Open-field test, a 2 × 1

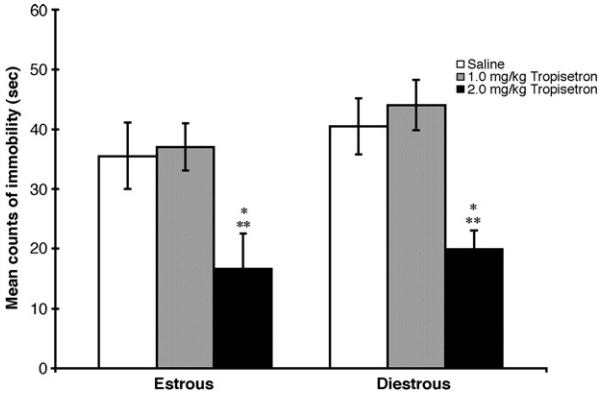

Fig. 1. Effect of tropisetron on immobility behavior of estrous and diestrous female rats. Figure above shows the mean number of counts (±S.E.) for immobility behavior recorded during the 5-min test session. Rats were treated with either 0.9% saline, or 1.0 mg/kg tropisetron or 2.0 mg/kg tropisetron 30 min prior to the FST exposure. The numbers of animals in each group are as follows, estrous saline ($n=6$), estrous 1.0 mg/kg tropisetron ($n=6$), estrous 2.0 mg/kg tropisetron ($n=6$), diestrous saline ($n=7$), diestrous 1.0 mg/kg tropisetron ($n=7$), diestrous 2.0 mg/kg tropisetron ($n=6$). Single asterisk indicate significant differences from the saline control. Double asterisks indicate significant differences from the 1.0 mg/kg treated-group.

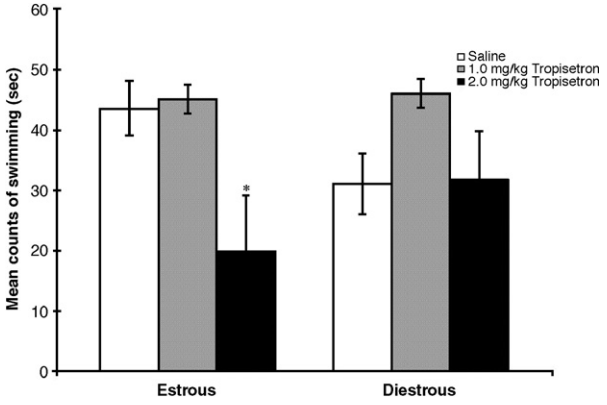

Fig. 2. Effect of tropisetron on swimming behavior of estrous and diestrous female rats. Figure above shows the mean number of counts (±S.E.) for swimming behavior of rats recorded during the 5-min test session. Rats were treated with 0.9% saline, or 1.0 mg/kg tropisetron or 2.0 mg/kg tropisetron. The numbers of animals in each group are estrous saline ($n=6$); estrous 1.0 mg/kg tropisetron ($n=6$); estrous 2.0 mg/kg tropisetron ($n=6$); diestrous saline ($n=7$); diestrous 1.0 mg/kg tropisetron ($n=7$) and diestrous 2.0 mg/kg tropisetron ($n=6$). Single asterisk indicates significant difference from the saline control.

factor ANOVA was used to analyze the effects of tropisetron treatment on total number of crossings made by both the estrous and diestrous rats. For all, an alpha levels of 0.05 was considered as being statistically significant. Statistical reference was Zar (1996).

3. Results

3.1. Immobility behavior

The effects of the 5-HT3 receptor antagonist, tropisetron, on immobility are shown in Fig. 1. There were significant treatment effects on immobility in both estrous and diestrous female rats (ANOVA [$F_{2,32}=13.34$, $P=0.001$]), but the main effect of stage

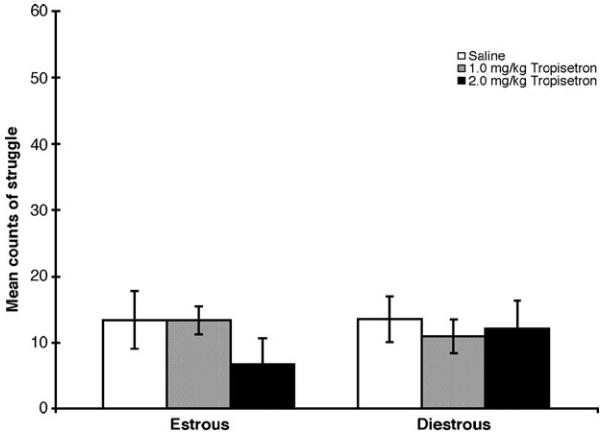

Fig. 3. Effect of tropisetron on struggling behavior of estrous and diestrous female rats. Figure above shows the mean number of counts (±S.E.) for struggling behavior of rats recorded during the 5-min test session. Rats were treated with 0.9% saline, or 1.0 mg/kg tropisetron or 2.0 mg/kg tropisetron. The numbers of animals in each group are estrous saline ($n=6$); estrous 1.0 mg/kg tropisetron ($n=6$); estrous 2.0 mg/kg tropisetron ($n=6$); diestrous saline ($n=7$); diestrous 1.0 mg/kg tropisetron ($n=7$) and diestrous 2.0 mg/kg tropisetron ($n=6$).

Table 1
Effect of saline or 2.0 mg/kg tropisetron on general locomotor activity

Stage	Treatment	Crosses
Estrous	Saline ($n=9$)	102.44±9.01
	2.0 mg/kg Tropisetron ($n=9$)	89.44±16.18
Diestrous	Saline ($n=9$)	121.78±11.46
	2.0 mg/kg Tropisetron ($n=8$)	88.13±13.41

Data are presented as mean (±SE) number of crosses in the Open-field test apparatus in estrous and in diestrous female rats.

of the cycle was not significant (ANOVA [$F_{1,32}=1.80$, $P=0.19$]). There was also no interaction between treatment and stage (ANOVA [$F_{2,32}=0.08$, $P=0.93$]). Both estrous and diestrous rats responded to 2.0 mg/kg of tropisetron by showing a significant decline in immobility (Tukey's q 40, $3\geq3.44$, $P\leq0.05$). The lower dose of the drug did not differ significantly from saline-treated rats. However, estrous rats treated with 2.0 mg/kg tropisetron was significantly different from the saline control and the 1.0 mg/kg tropisetron-treated estrous rats (Tukey's q 40, $3\geq3.44$, $P\leq0.05$). Similarly, the diestrous rats treated with 2.0 mg/kg tropisetron was also significantly different from the saline control and the 1.0 mg/kg tropisetron-treated diestrous rats (Tukey's q 40, $3\geq3.44$, $P\leq0.05$).

3.2. Swimming behavior

Similar to the effects of tropisetron on immobility behavior, there was a significant effect of treatment (ANOVA [$F_{2,32}=5.88$, $P=0.01$]), but no effects of stage (ANOVA [$F_{1,32}=0.001$, $P=0.98$]) or their interaction (ANOVA [$F_{2,32}=2.23$, $P=0.12$]) on swimming behavior (Fig. 2). Only the estrous rats treated with 2.0 mg/kg of tropisetron showed a significant decline in swimming (Tukey's q 40, $3\geq3.44$, $P\leq0.05$). No dose of tropisetron had any significant effects in the diestrous rats. Although the saline-treated distrous rats appeared to show lower counts of swimming compared to the saline-treated estrous rats, Tukey's test revealed no significant differences between the two groups ($P>0.05$).

3.3. Struggling behavior

There were no significant effects of treatment (ANOVA [$F_{2,32}=0.68$, $P=0.51$]), stage (ANOVA [$F_{1,32}=0.12$, $P=0.73$]), or their interaction (ANOVA [$F_{2,32}=0.63$, $P=0.53$]) on struggling behavior following tropisetron treatment (Fig. 3). No dose of drug examined caused any changes in struggling behavior in either the estrous or in the diestrous rats.

3.4. Open-field test

As shown in Table 1, treatment with 2.0 mg/kg tropisetron failed to alter locomotor activity in any group of rat. There were no significant effects of either treatment (ANOVA [$F_{1,31}=3.33$, $P=0.08$]) or stage (ANOVA [$F_{1,31}=0.49$, $P=0.08$]) or their interaction (ANOVA [$F_{1,31}=0.65$, $P=0.425$]) on the activity of rats.

4. Discussion

Consistent with previous findings that 5-HT3 receptor antagonists reduce immobility behavior in male rats (Nakagawa et al., 1998), treatment with tropisetron also reduced immobility in female rats exposed to the FST. Furthermore, the effects of tropisetron on immobility were evident after a single treatment. However, the decline in immobility after tropisetron treatment did not vary according to the estrous cycle of the female rats. Since estrogen is thought to act as a non-competitive antagonist at the 5-HT3 receptors (Oz et al., 2002; Wetzel et al., 1998), we had anticipated that the estrous rats with higher estrogen levels than the diestrous rats (Freeman, 1988) would accentuate the 5-HT3 blocking effects of tropisetron and show a greater decline in immobility. In a study evaluating the impact of FST in female rats, Shors et al. (1999), reported that plasma estrogen levels were increased in female rats from all stages of the estrous cycle after exposure to the FST. Consequently, in our experiment, a stress-induced increase in estrogen levels in both groups of rats may have eliminated any cycle-dependent difference in the response to tropisetron.

Treatment with estrogen in ovariectomized female rats has been reported to decrease immobility (Estrada-Camarena et al., 2003; Rachman et al., 1998). Furthermore, female rats in low estrogen stages of the cycle (e.g. diestrus) display greater immobility than at other stages of the cycle (e.g. proestrus) (Frye and Walf, 2002; Marvan et al., 1996, 1997). However, not all investigators agree with such findings. Alonso et al. (1991) reported no differences in immobility of female rats from different stages of the cycle. In agreement with Alonso et al. (1991), our diestrous rats did not display significantly lower immobility than the estrous rats. However, the experimental design used in our study included behavior from the diestrous rats, but did not include behaviors from rats in the proestrous stage. Thus, inclusion of data from proestrous rats may have impacted the findings of our study.

Exposure to FST increases corticosterone levels in both male and female rats (Connor et al., 1997; Shors et al., 1999). Corticosterone has been reported by some investigators to increase immobility (Baez and Volosin, 1994; Hill et al., 2003); however, in other studies corticosterone was reported to decrease immobility (Broto et al., 2001). Unlike restraint stress, which produces a greater elevation of corticosterone in estrous than in diestrous females (Figueiredo et al., 2002), FST-induced elevations in corticosterone are not estrous cycle-dependent (Shors et al., 1999). Consequently, similar increases in corticosterone levels across all stages of the cycle, may explain the lack of a significant difference in immobility behavior between estrous and the diestrous rats.

In our study when swimming behavior was examined in estrous and in diestrous rats, no estrous cycle differences were observed. Estrogen-treatment in ovariectomized female rats increases swimming (Estrada-Camarena et al., 2003; Rachman et al., 1998). Therefore, we had anticipated the estrous rats with higher estrogen to display a greater swimming behavior than diestrous rats. However, our data did not support such findings. FST-induced elevations in estrogen levels across all groups may have eliminated any estrous cycle differences on swimming.

Compounds that act as antidepressants, reduce indices of depressive behavior in the FST by causing a decrease in immobility and/ or an increase in swimming (Detke et al., 1995; Estrada-Camarena et al., 2003, 2004; Frye and Walf, 2002; Hernandez-Molina and Tellez-Alcantara, 2001; Hernandez-Molina et al., 2005). However, in our study tropisetron-treated estrous rats displayed a decrease and not an increase in swimming. Although such findings were contrary to expectations, studies implicate that drugs that enhance 5-HT neurotransmission increase active behavior such as swimming (Detke et al., 1995; Estrada-Camarena et al., 2004). Since tropisetron is an antagonist at the 5-HT3 receptors, the decrease in swimming in the current study may be due to decreased 5-HT neurotransmission. Furthermore, the decreased swimming was evident in the estrous and not in the diestrous rats. As previously mentioned estrogen also acts as an antagonist at the 5-HT3 receptors (Oz et al., 2002; Wetzel et al., 1998). In estrous rats the presence of higher levels of estrogen may have further antagonized the 5-HT3 receptors and consequently, accentuated the effects of tropisetron on swimming.

As apparent from the Open-field test (OFT), the decline in swimming was not due to tropisteron's effect on general locomotor activity. The OFT is used to rule out non-specific actions of antidepressants. Antidepressants are generally reported to either decrease, or cause no changes in the general activity in the OFT (Detke et al., 1995; Estrada-Camarena et al., 2004; Porsolt et al., 1977). Tropisteron did not reduce general activity in either estrous or diestrous rats.

When another active behavior, struggling, was evaluated in the estrous or the diestrous rats, there were no effects of tropisetron. Such findings may be expected, since studies implicate that drugs that mainly enhance noradrenergic transmission increase struggling behavior in the FST (Detke et al., 1995; Estrada-Camarena et al., 2004). Drugs acting on the 5-HT system are mainly thought to have an effect on swimming behavior in the FST (Detke et al., 1995; Estrada-Camarena et al., 2004).

Although we have emphasized the role of estrogen, we cannot rule out the contribution of progesterone in the FST. Progesterone levels fluctuate during the estrus cycle (Freeman et al., 1988). Similar to estrogen, progesterone also antagonizes the actions of the 5-HT3 receptors (Oz et al., 2002; Wetzel et al., 1998). More specifically, treatments with progesterone or progesterone metabolites influence behaviors in the FST by causing a decrease in immobility (Frye and Walf, 2002; Hernandez-Molina et al., 2005; Martinez-Mota et al., 1999). Thus the synchronized actions of both estrogen and progesterone on the 5-HT3 receptors may influence the behaviors in the FST. Although females in estrous and diestrous would differ in plasma concentrations of progesterone, unlike estrogen, exposure to the FST does not elevate progesterone levels (Shors et al., 1999). Therefore, any cycle-dependent contribution of progesterone on behaviors displayed during FST may have been overridden by an FST-induced elevation of estrogen. Since we used intact female rats in our study, we were unable to further dissect the individual contributions of estrogen/progesterone on 5-HT3 receptors. Future studies, examining the effects of tropisetron in hormone-treated ovariectomized rats may provide

more specific information regarding the interaction between gonadal hormones and 5-HT3 receptors. Furthermore, in the current study we evaluated the effects of only two doses of tropisetron on FST. Evaluating the effects of other doses of tropisetron may provide further insights into the mechanism of drug action in female rats.

In summary, a single treatment with the 5-HT3 receptor antagonist, tropisetron, reduced immobility and swimming, without changing struggling behavior of female rats exposed to the FST. In spite of recognized effects of gonadal hormones on 5-HT3 receptor functions, the effects of tropisetron on immobility did not differ between the estrous and diestrous females. However, tropisetron was effective in reducing swimming only in the estrous rats. Thus different mechanisms or different types of interactions between gonadal hormones and 5-HT3 receptors may be involved in the regulation of immobility and swimming behaviors in adult female rats exposed to the FST.

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