Published in final edited form as:

Horm Behav. 2010 July ; 58(2): 290–296. doi:10.1016/j.yhbeh.2010.03.003.

ROLE OF 5-HT_{1A} RECEPTORS IN FLUOXETINE-INDUCED LORDOSIS INHIBITION

Jutatip Guptarak¹, Jhimly Sarkar², Cindy Hiegel, and Lynda Uphouse

Department of Biology, Texas Woman's University, Denton, Texas 76204

Abstract

The selective serotonin reuptake inhibitor (SSRI), fluoxetine (Prozac®), is an effective antidepressant that is also prescribed for other disorders (e.g. anorexia, bulimia, and premenstrual dysphoria) that are prevalent in females. However, fluoxetine also produces sexual side effects that may lead patients to discontinue treatment. The current studies were designed to evaluate several predictions arising from the hypothesis that serotonin 1A (5-HT_{1A}) receptors contribute to fluoxetine-induced sexual dysfunction. In rodent models, 5-HT_{1A} receptors are potent negative modulators of female rat sexual behavior. Three distinct experiments were designed to evaluate the contribution of 5-HT_{1A} receptors to the effects of fluoxetine. In the first experiment, the ability of the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY100635), to prevent fluoxetine-induced lordosis inhibition was examined. In the second experiment, the effects of prior treatment with fluoxetine on the lordosis inhibitory effect of the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT), was studied. In the third experiment, the ability of progesterone to reduce the acute response to fluoxetine was evaluated. WAY100635 attenuated the effect of fluoxetine; prior treatment with fluoxetine decreased 8-OH-DPAT's potency in reducing lordosis behavior; and progesterone shifted fluoxetine's dose response curve to the right. These findings are consistent with the hypothesis that 5-HT_{1A} receptors contribute to fluoxetine-induced sexual side effects.

Keywords

lordosis; serotonin; sexual dysfunction; SSRIs; ovariectomized rat; antidepressant; progesterone; hormonal priming

Introduction

In addition to its use as an effective antidepressant, fluoxetine (Prozac®) is prescribed for other disorders (e.g. anorexia, bulimia nervosa, and menstrual dysphoria) that are prevalent in women (Rossi et al., 2004; Simpson and Noble, 2000). Unfortunately, sexual dysfunction often occurs after fluoxetine treatment and is one of the causes for patients stopping

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Corresponding Author: Dr. Lynda Uphouse, Department of Biology, Texas Woman's University, Denton, Texas 76204, Phone: 940-898-2356, Fax: 940-898-2382, LUphouse@mail.twu.edu.

¹Current Address: Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555

²Current Address: Neuroscience Program, University of Illinois at Urbana-Champaign, 2001 S. Lincoln Ave., Urbana, IL, 61801

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medication (Hartmann, 2007). Since multiple factors and depression, itself, can contribute to sexual dysfunction, the investigation of fluoxetine-induced sexual dysfunction is complicated; in addition, results may be confounded with factors such as drug use or co-morbid medical conditions that may impact sexual activity (Hartmann, 2007; Michelson et al., 2001; Segraves, 2007). Furthermore, outcomes can vary with the investigator's definition of sexual dysfunction. Patients may show decline in some phase of sexual activity without exhibiting dysfunction in every phase of the sexual response cycle (e.g. desire, arousal, and orgasm) (Clayton et al., 2006). Thus, the mechanisms responsible for fluoxetine-induced sexual dysfunction have been difficult to determine. Animal models, therefore, may be especially valuable tools for dissecting mechanisms responsible for SSRI-induced sexual dysfunction.

In rodent models, fluoxetine can interrupt normal estrous cyclicity and reduce female sexual behavior (Uphouse et al., 2006). The lordosis reflex (arching of the back made by a sexually receptive female rat in response to a mount by the male) has been the most examined aspect of female rodent sexual behavior that is routinely reported to decline following treatment with fluoxetine (Frye et al., 2003; Matuszczyk et al., 1998; Sarkar et al., 2008; Uphouse et al., 2006). Although the mechanisms responsible for fluoxetine's reduction of lordosis behavior are not known, fluoxetine's block of the serotonin transporter and consequent elevation of extracellular serotonin (5-HT) is a likely candidate. 5-HT is well known for its inhibitory role in female sexual behavior and hypothalamic 5-HT_{1A} receptors play a dominant role in the lordosis inhibition. Infusion into the ventromedial nucleus of the hypothalamus (VMN) or systemic injection of 5-HT_{1A} receptor agonists inhibits the lordosis reflex (Mendelson, 1992; Uphouse, 2000) and this inhibitory effect is blocked by 5-HT_{1A} receptor antagonists (Snoeren et al., 2009; Uphouse et al., 1996; Uphouse and Wolf, 2004). Since fluoxetine leads to an increase in extracellular 5-HT (Perry and Fuller, 1992; Tao et al., 2002) and consequent activation of 5-HT receptors, increased activation of 5-HT_{1A} receptors would be expected to occur and to inhibit lordosis behavior. If so, prior treatment with the 5-HT_{1A} receptor antagonist, WAY100635, should attenuate fluoxetine's effect. Although WAY100635 was reported to attenuate the effect of fluoxetine on noncontact penile erection in male rats (Sukoff Rizzo et al., 2009), effects on female sexual behavior after fluoxetine have not been reported. In the following experiments, the effect of WAY100635 on fluoxetine's reduction of lordosis behavior was examined. Two conditions that reduce effects of 5-HT_{1A} receptor agonists were also investigated.

Progesterone reduces 8-OH-DPAT's potency as an inhibitor of lordosis behavior (Truitt et al., 2003). In the following studies, the effects of hormonal priming with estrogen or with progesterone on the response to fluoxetine was examined. Similarly, because repeated fluoxetine treatment desensitizes 5-HT_{1A} receptors (Li et al., 1996), the effects of 9 days of fluoxetine on the response to 8-OH-DPAT was examined. Although desensitization of somatodendritic 5-HT_{1A} autoreceptors of the dorsal raphe occurs rapidly in response to fluoxetine treatment, 5-HT_{1A} receptors in terminal fields are slower to desensitize (Dawson and Nguyen, 2000; Hensler, 2003). However, seven to ten days of daily fluoxetine treatment reduced the neuroendocrine response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Li et al., 1996; Van de Kar et al., 2002). Since hypothalamic 5-HT_{1A} receptors contribute to serotonin-mediated inhibition of lordosis behavior, it was expected that 9 days of fluoxetine would reduce the lordosis-inhibitory effects of a 5-HT_{1A} receptor agonist. Findings from all three approaches are consistent with the hypothesis that the 5-HT_{1A} receptors may be critical mediators of the negative effects of fluoxetine on female rat sexual behavior.

Materials and Methods

Materials

Estradiol benzoate (EB), progesterone (P), sesame seed oil, the serotonin reuptake inhibitor, [$\pm$]-N-methyl- γ -[4-(trifluoromethyl)-phenoxy]benzenepropanamine (fluoxetine), the 5-HT_{1A} receptor agonist, ($\pm$)-8-hydroxy 2-(di-n-propylamino) tetralin (8-OH-DPAT), and the 5-HT_{1A} receptor antagonist, *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100635), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Isoflurane (AErrane[®]) and suture materials were purchased from Henry Schein (Melville, NY). Food (8604 Harlan Teklad rodent diet) was purchased from Harlan Teklad (Madison, WI). All other supplies were purchased from Fisher Scientific (Houston, TX).

General Methods

Animals and housing conditions—Female, Fischer inbred rats (CDF-344) were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed 2 or 3 per cage in polycarbonate shoebox cages (45.72 × 24.13 × 2.59 cm) in a housing room maintained at 25 °C and 55% humidity with a 12:12 hr light-dark cycle (lights on from 12:00 AM to 12:00 PM). Food and water were available *ad lib*.

Surgical Procedures—Two weeks after arrival, rats were bilaterally ovariectomized under AErrane[®] anesthesia as previously described (White and Uphouse, 2004), and experimental procedures began two weeks later. All procedures were in accordance with PHS policy and were approved by the IACUC at Texas Woman's University.

Behavioral testing procedures—Behavioral testing took place during the dark phase of the light-dark cycle under dim red lighting to facilitate the experimenter's ability to observe the rats. Females were pretested for sexual receptivity and proceptivity by placing the female in the home cage of a sexually experienced male and observing her behavior for ten mounts. After the pretest, the female was injected with the appropriate drug(s) and returned to the male's cage. Sexual behavior was monitored as previously described (White and Uphouse, 2004). Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (e.g. number of lordosis responses by the female divided by the number of mounts by the male). Lordosis quality was recorded on a scale of 1 to 4 as previously described (White and Uphouse, 2004). The number of incidences of proceptivity, defined as hop/dart sequences, was recorded.

Handling procedures—On the day of the estrogen injection, each rat was picked up, held for 15 sec and turned as in preparation for an intraperitoneal (ip) injection. This procedure was then repeated and constituted a handling session. After all rats in the cage had received a handling session, the procedure was repeated until each rat had received 5 handling sessions. The entire process was repeated the following day.

Statistical analyses—Data were analyzed with SuperAnova 1.11 or SPSS 17. L/M ratios and lordosis quality data were grouped into the pretest and 5-min intervals after treatment. In the first experiment, data were compared by 1-way repeated measures ANOVA with drug (WAY100635 or vehicle) as the independent factor. In the second and third experiments, data were analyzed by 2-factor repeated measures ANOVA with time after infusion as the repeated measure and prior treatment and dose of drug as independent factors. Time-dependent differences, within treatment, were compared to the pretest with Dunnett's test; differences between groups, within time intervals, were compared by Tukey's test.

Proportional data were evaluated with Chi-square procedures or Fisher's Exact Test. An alpha level of 0.05 was required to reject the null hypothesis.

Specific Methods

Experiment 1: The effect of the 5-HT_{1A} receptor antagonist, WAY100635, on fluoxetine-induced lordosis inhibition—An N of 10 rats was used for each treatment. Fourteen days after ovariectomy, rats were injected ip with 1 ml/kg vehicle (distilled water) for 9 days. On day 8 of these injections, between 9:00 and 9:30 am, females received 10 µg estradiol benzoate followed 48 hr later with 500 µg progesterone. Hormones were dissolved in sesame seed oil and injected subcutaneously (sc) in a volume of 0.1 ml per rat. Four to six hours after progesterone, rats were pretested for sexual behavior. Rats were injected with either 1 ml/kg saline or 1 mg/kg WAY100635 (in saline, 1 ml/kg). Fifteen min later, rats were injected with 15 mg/kg fluoxetine in distilled water (1.5 ml/kg). Sexual behavior was tested 30 min later and continued for 15 consecutive min. The experimental protocol is shown in Figure 1. The dose of 15 mg/kg fluoxetine was chosen on the basis of a previous study (Sarkar et al., 2008) and on a preliminary dose-response analysis of the lordosis-inhibiting effects of fluoxetine. A dose of 1 mg/kg WAY100635 was based on prior literature (Crane et al., 2007; Tohyama et al., 2001) with WAY100635, evidence that a comparable dose was required to block postsynaptic 5-HT_{1A} receptors (Vicentic et al., 1998) and our earlier work with intracranial WAY100635 and lordosis behavior (Uphouse and Wolf, 2004).

Experiment 2: The effect of prior treatment with fluoxetine on the response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT—Ten to fourteen days after ovariectomy, thirty females were injected ip daily for 9 days with 1 ml/kg vehicle (distilled water), and 29 females were injected daily with 10 mg/kg fluoxetine (1 ml/kg). The hormone treatment occurred on days 8 and 10 as described for experiment 1. Four to six hours after the progesterone injection, rats were pretested for sexual receptivity. Immediately after the pretest, rats received an ip injection of 0, 25, 50, 75, or 100 µg/kg of 8-OH-DPAT. Females were placed immediately back into the male's cage and testing was continued for 30 consecutive min.

Experiment 3: The effect of progesterone on the response to fluoxetine—Two weeks after ovariectomy, rats were handled and injected ip with 1 ml/kg vehicle (saline) for 3 days. On the first day of handling, rats were injected sc with 10 µg estradiol benzoate. Rats received progesterone (EP rats) or the sesame seed oil vehicle (EO rats) 46 to 48 hr after estrogen. Four to six hours after progesterone or vehicle, rats were pretested for sexual behavior. After 10 mounts by the male, the female was injected ip with 0, 2.5, 5.0, 7.5, 10 or 15 mg/kg fluoxetine in distilled water (1 ml/kg except for 15 mg/kg = 1.5 ml/kg). The female was returned to the home cage for 30 min. Sexual behavior was monitored for 15 consecutive min thereafter.

Results

Experiment 1

The first experiment was designed to test the hypothesis that the 5-HT_{1A} antagonist, WAY100635, would attenuate the acute effect of fluoxetine on lordosis inhibition. As shown in Figure 2, fluoxetine significantly reduced the L/M ratio and this decrease was attenuated by WAY100635 ($F_{1, 18} = 11.21$, $P = 0.005$). There was also a significant effect of time after treatment ($F_{3, 54} = 24.88$, $P = 0.0001$) and a significant time by treatment interaction ($F_{3, 54} = 5.95$, $P = 0.002$). In the absence of WAY100635, fluoxetine-treated rats were significantly different from their pretest at every test interval (Dunnett's, all $q_{54, 4}$

5.84, $P = 0.05$). With WAY100635, L/M ratios were significantly different from the pretest only at the first 5 min interval (Dunnett's $q_{54, 4} = 2.90$, $P = 0.05$), and the degree of inhibition was small relative to the vehicle control. Rats treated with WAY100635 plus fluoxetine differed significantly from rats treated with saline plus fluoxetine at each test interval (Tukey's, all $q_{54, 2} = 4.85$, $P = 0.05$).

L/M ratios of 3 of the 10 saline-treated rats fell to zero at some point during testing so that lordosis quality could not be assessed. For the remaining rats, there was a modest time-dependent decline in lordosis quality ($F_{3, 45} = 6.63$, $P = 0.001$, data not shown), but neither the treatment nor the time by treatment interaction was significant ($P > 0.05$). The mean $\pm$ S.E. lordosis quality/interval for saline and WAY100635-treated rats, respectively, was 2.61 ± 0.12 and 2.80 ± 0.48 . The average number of mounts for saline and WAY100635-treated rats, respectively, was 7.95 ± 0.45 and 9.65 ± 0.64 . There were no significant effects of the pretreatment on number of mounts received by the females (all $P > 0.05$).

Prior to fluoxetine, every rat showed evidence of proceptivity. In saline-pretreated rats, only 2/10 rats remained proceptive after fluoxetine while 7/10 rats pretreated with WAY100635 remained proceptive. Thus, proceptivity was reduced by fluoxetine in the saline (Fisher's Exact Test, $P = 0.001$), but not in the WAY100635, pretreated rats (Fisher's Exact Test, $P > 0.05$).

Experiment 2

The second experiment was designed to test the hypothesis that prior treatment with fluoxetine would attenuate lordosis inhibition in response to a 5-HT_{1A} receptor agonist. There was a dosedependent effect of 8-OH-DPAT on sexual receptivity (Fig. 3) and prior fluoxetine treatment shifted the dose response curve to the right. Females subchronically treated with distilled water and injected on the 10th day with doses of 8-OH-DPAT of at least 50 $\mu\text{g}/\text{kg}$ showed near maximal reduction in the lordosis reflex within 10 min of injection. In contrast, for rats pretreated with fluoxetine, a dose of 100 $\mu\text{g}/\text{kg}$ 8-OH-DPAT was required for maximal inhibition of lordosis behavior and lower doses of the drug (while producing some inhibition of the L/M ratio) were less effective in fluoxetine-pretreated than in water-pretreated rats (Fisher's Exact Test, $P = 0.05$). Therefore, for the L/M ratio, there were significant main effects of type of prior treatment ($F_{1, 49} = 21.32$, $P = 0.0001$), dose of 8-OH-DPAT ($F_{4, 49} = 52.24$, $P = 0.0001$) and their interaction ($F_{4, 49} = 7.78$, $P = 0.0001$). Over time, L/M ratios declined ($F_{6, 294} = 58.56$, $P = 0.0001$) and time interacted significantly with type of pretreatment ($F_{6, 294} = 4.61$, $P = 0.0002$) and with dose of 8-OH-DPAT ($F_{24, 294} = 5.50$, $P = 0.0001$). The 3-way interaction was not significant ($P > 0.05$).

For water-pretreated rats, all doses of 8-OH-DPAT inhibited lordosis behavior by 10 to 15 min and thereafter (all Dunnett's $q_{294, 10} = 2.69$, $P = 0.05$), and, for water-pretreated rats, there were no significant differences among rats treated with 50, 75 or 100 $\mu\text{g}/\text{kg}$ 8-OH-DPAT. For fluoxetine-pretreated rats, doses of 75 and 100 $\mu\text{g}/\text{kg}$ were required to produce significant reductions in L/M ratios relative to the pretest scores. For these two doses, L/M ratios were significantly less than the pretest by at least 10 min. However, for lower doses of 8-OH-DPAT, there was minimal inhibition of lordosis in fluoxetine-pretreated rats. For doses of 50 and 75 $\mu\text{g}/\text{kg}$ 8-OH-DPAT, fluoxetine and water-pretreated rats differed significantly at 10 min and thereafter (all Tukey's $q_{249, 2} = 2.77$, $P = 0.05$, Fig. 3B).

A large proportion of rats (18/30) pretreated with distilled water and then injected with 8-OH-DPAT had L/M ratios of zero during the testing period so that lordosis quality could not be examined. Prior treatment with fluoxetine significantly reduced the number (5/29) of rats showing zero L/M ratios (Fisher's Exact Test, $P = 0.001$). Thus, lordosis quality could be statistically analyzed for all doses of 8-OH-DPAT except 100 μg in fluoxetine-pretreated

rats. For these fluoxetine-pretreated rats (Fig. 4), there was a significant effect of the 8-OH-DPAT dose ($F_{3, 19} = 5.56$, $P = 0.01$) and time after injection ($F_{6, 144} = 6.55$, $P = 0.0001$) on lordosis quality, but the time by dose interaction was not significant ($P > 0.05$). Small, but significant declines in quality were evident in rats treated with either 50 or 75 $\mu\text{g/kg}$ (Dunnett's $q_{114, 7} = 2.64$, $P = 0.05$).

8-OH-DPAT dose-dependently reduced the number of rats showing proceptivity (Chi square = 17.31, $df = 4$, $P = 0.002$), due primarily to a near absence (12.5%) of proceptivity in rats injected with 100 μg 8-OH-DPAT. However, there was no effect of prior treatment (Fischer Exact Test, $df = 1$, $P > 0.05$). For water and fluoxetine pretreatments, 43.3% and 38.0% of rats showed proceptivity after 8-OH-DPAT treatment.

The average number of mounts per interval for rats pretreated with water or fluoxetine, respectively, was 7.67 ± 0.32 and 8.44 ± 0.29 , and there were no significant effects of either prior treatment or dose of 8-OH-DPAT. However, across all groups, there was a decline in number of mounts over the 30 min test period ($F_{6, 294} = 5.90$, $P = 0.05$) and this was slightly accentuated by 8-OH-DPAT (time by dose, $F_{24, 294} = 1.74$, $P = 0.02$; data not shown). Neither the time by prior treatment nor the 3-way interaction was significant ($P > 0.05$).

Experiment 3

In the third experiment, progesterone was shown to attenuate fluoxetine-induced lordosis inhibition (ANOVA for hormone treatment, $F_{1, 61} = 8.98$, $P = 0.005$; Table 1). There was also a significant effect of dose of fluoxetine ($F_{5, 61} = 14.87$, $P = 0.0001$) as well as a significant effect of time ($F_{3, 183} = 31.23$, $P = 0.0001$) and the time by fluoxetine dose interaction ($F_{15, 183} = 5.29$, $P = 0.0001$). EO and EP rats differed in a fluoxetine-dose-dependent manner so that the two groups differed significantly at doses of 5 and 7.5 mg/kg fluoxetine (Tukey's $q_{61, 6} = 4.16$, $P = 0.05$), but not at 10 or 15 mg/kg .

Most rats (11/12) treated with 15 mg/kg fluoxetine had L/M ratios of zero at some point during testing and, at this dose, EO and EP rats did not differ in the proportion of rats showing zero L/M ratios (Fisher's Exact Test, $P > 0.05$); therefore, this dose was excluded from analysis of lordosis quality. For the remaining groups, there was a small, but significant, effect of hormonal treatment ($F_{1, 38} = 7.80$, $P = 0.01$; mean $\pm$ S.E. for EO and EP rats, respectively, = 1.75 ± 0.04 and 2.89 ± 0.03) and dose of fluoxetine ($F_{4, 38} = 5.62$, $P = 0.002$; mean $\pm$ S.E. ranged from 2.90 ± 0.04 to 2.62 ± 0.08) on lordosis quality, but time after injection was not significant ($P > 0.05$). Although there was a significant interaction between time and dose of fluoxetine ($F_{114, 12} = 2.64$, $P = 0.005$), none of the interactions with hormone treatment were significant (all $P > 0.05$).

Since EO rats generally show little proceptivity, an evaluation of the effect of progesterone on fluoxetine-reduced proceptivity was not meaningful. In EP rats, there was some indication that fluoxetine reduced proceptivity at the higher doses. However, the overall amount of proceptivity in this experiment was relatively low so that there was not a significant effect of dose ($P > 0.05$).

The average number of mounts per interval for EO and EP rats, respectively, was 6.54 ± 0.30 and 6.97 ± 0.27 . There was a decline in number of mounts over the 15 min testing period ($F_{183, 3} = 40.04$, $P = 0.0001$), but no other effects were significant ($P > 0.05$).

Discussion

Three major findings emerged from these studies: (1) The 5-HT_{1A} receptor antagonist, WAY100635, attenuated the ability of an acute fluoxetine injection to inhibit lordosis

behavior; (2) subchronic treatment with fluoxetine reduced the potency of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, as an inhibitor of lordosis behavior; and (3) progesterone, previously reported to attenuate effects of 8-OH-DPAT (Truitt et al., 2003), reduced the potency of an acute fluoxetine treatment in inhibiting lordosis behavior. These findings are consistent with the hypothesis that increased activation of 5-HT_{1A} receptors contributes to fluoxetine's sexual side effects.

These are the first studies to document an attenuating effect of WAY100635 on fluoxetine-induced female rat sexual behavior and are consistent with prior studies in male rats (Sukoff Rizzo et al., 2009; Sukoff Rizzo et al., 2008). WAY100635 is a relatively selective compound with high affinity for 5-HT_{1A} receptors (Cliffe, 2000; Martel et al., 2007), is a potent antagonist of 5-HT_{1A} receptor agonist-mediated effects (Castro et al., 2008; Crane et al., 2007; Invernizzi et al., 1996) and can effectively block effects of 8-OH-DPAT on female rat lordosis behavior (Snoeren et al., 2009; Uphouse and Wolf, 2004).

Because both drugs were delivered systemically, the site of action for WAY100635 cannot be determined from these studies. Fluoxetine treatment elevates extracellular 5-HT in the dorsal raphe and in terminal fields of 5-HT neurons, including the hypothalamus (Auerbach et al., 1989; Malagie et al., 1995; Tao et al., 2002). At the dorsal raphe, antagonism of 5-HT_{1A} receptors should amplify effects of fluoxetine on extracellular 5-HT (Ago et al., 2003; Dawson and Nguyen, 2000; Tao et al., 2002). Since further elevation in extracellular 5-HT would be expected to enhance, rather than attenuate, the lordosis-inhibitory effects of fluoxetine, it is unlikely that WAY100635's antagonism of somatodendritic 5-HT_{1A} receptors is responsible for the current findings. However, at terminal fields, WAY100635, by blocking 5-HT_{1A} receptors, would not only reduce 5-HT_{1A} mediated-signals but could increase the probability for activation of other 5-HT receptor subtypes, such as 5-HT_{1B}, 5-HT_{2A/2C} and 5-HT₃, which may increase lordosis responding (Maswood et al., 1997; Maswood et al., 1998; Uphouse et al., 1994; Wolf et al., 1999; Wolf et al., 1998). Such an obvious alteration in the balance between lordosis inhibitory and facilitatory 5-HT receptors would undoubtedly reduce effects of fluoxetine.

Thus, the most parsimonious explanation for the findings of the first experiment is that fluoxetine's elevation of extracellular 5-HT increases activation of 5-HT_{1A} receptors in terminal fields that then leads to inhibition of lordosis behavior. WAY100635, by blocking 5-HT_{1A} receptors, attenuates the effect of this fluoxetine-induced increase in extracellular 5-HT. The findings of the third experiment, where progesterone reduced fluoxetine's potency for inhibiting lordosis behavior, are consistent with this explanation. Progesterone shifted the dose response curve for fluoxetine to the right in a manner comparable to that previously reported for the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Truitt et al., 2003). Progesterone's ability to reduce the effects of fluoxetine is particularly interesting since, in intact Fischer females, fluoxetine disrupts the normal estrous cycle and associated sexual behavior and also reduces plasma levels of progesterone (Uphouse et al., 2006).

Progesterone reduces extracellular 5-HT in various brain areas, including the hypothalamus (Farmer et al., 1996; Maswood et al., 1999), and reduced hypothalamic 5-HT occurs coincident with the emergence of female rat sexual receptivity (Farmer et al., 1996; Gundlah et al., 1998; Maswood et al., 1999). The decline in hypothalamic extracellular 5-HT that occurs during proestrus (the stage of the estrous cycle associated with sexual receptivity) is thought to result from a preovulatory surge of progesterone. The SSRI-induced increase in extracellular 5-HT occurs as a consequence of blocking the serotonin transporter. If, as previously suggested, progesterone reduces turnover and release of 5-HT (Farmer et al., 1996; Renner et al., 1987), the SSRI-induced increase in extracellular 5-HT would also be lessened. There is some evidence to suggest that progesterone may increase 5-HT_{1B}

receptors (Frankfurt et al., 1994); terminal 5-HT_{1B} receptors modulate release of 5-HT (Daws et al., 2000); and a 5-HT_{1B} receptor antagonist can potentiate fluoxetine's elevation of extracellular 5-HT (Dawson and Nguyen, 2000). Thus, it is possible that progesterone may indirectly reduce the SSRI-induced elevation in extracellular 5-HT and thereby reduce activation of 5-HT_{1A} receptors.

Interestingly, it was recently reported that proestrous females showed a smaller response to the SSRI, fluvoxamine, following its administration to the hippocampus than did males or females in other stages of the estrous cycle (Benmansour et al., 2009). However, these authors attributed the effect primarily to estrogen rather than to progesterone. In the hypothalamus, progesterone (rather than estrogen) appears to be responsible for the proestrous decline in extracellular 5-HT (Farmer et al., 1996; Maswood et al., 1999).

Progesterone's reduction of 5-HT may be sufficient to account for the steroid's reduction in the effects of fluoxetine, but progesterone can facilitate lordosis behavior by several mechanisms. Progesterone's interaction with the classical intracellular progesterone receptor in the hypothalamus is both necessary and sufficient for the steroid to facilitate sexual behavior (Blaustein, 2008). However, neuroprogesterone is also important in regulation of reproductive functioning (Micevych and Sinchak, 2008; Micevych et al., 2008). Infusion of the progesterone metabolite, allopregnanolone, into the ventral tegmental area (VTA) positively affects female sexual behavior (Frye et al., 1998; Frye and Rhodes, 2006; Frye et al., 2006). Although fluoxetine alters the production of these progesterone metabolites in a complex manner, varying with brain region examined and duration of fluoxetine treatment (Griffin and Mellon, 1999; Pinna et al., 2009; Uzunov et al., 1996), VTA infusion of allopregnanolone reduces the effects of systemic fluoxetine on female sexual behavior (Frye et al., 2003). Allopregnanolone is recognized for its ability to enhance GABA_A receptor-mediated events (Barbaccia, 2004; Lambert et al., 2003; Rupprecht, 2003) and there is prior evidence that coactivation of GABA_A and 5-HT_{1A} receptors in the hypothalamus prevents 5-HT_{1A} receptor mediated inhibition of lordosis behavior (Guptarak et al., 2004). Thus, a potential role for progesterone metabolites should not be excluded. Moreover, since progesterone enhances the effect of estrogen in inducing lordosis behavior, we cannot rule out the possibility that higher doses of the drug are required to inhibit the robust sexual behavior of rats with the more optimal hormonal priming.

In a previous study, we reported that 9 days of fluoxetine treatment reduced the impact of fluoxetine on lordosis behavior when compared to effects of an acute treatment of the SSRI and suggested that a fluoxetine-induced desensitization of 5-HT_{1A} receptors could be responsible (Sarkar et al., 2008). The current findings that 9 days of fluoxetine shifted the dose-response curve for 8-OH-DPAT to the right is consistent with this earlier suggestion and with observations that daily fluoxetine reduced the neuroendocrine response to 5-HT_{1A} receptor agonist activation (Raap et al., 1999; Van de Kar et al., 2002). Mechanisms responsible for the reduced hypothalamic sensitivity to 5-HT_{1A} receptor agonists are not known, but may result from a fluoxetine-induced decline in the coupling of 5-HT_{1A} receptors to G-proteins (Li et al., 1996; Raap et al., 2000; Raap et al., 1999). Fluoxetine has a long half life (1–3 days) and its metabolite, norfluoxetine, has an even longer half life (Sanchez and Hyttel, 1999; Stokes and Holtz, 1997). Therefore, we cannot rule out the possibility that there was a direct interaction between fluoxetine (or norfluoxetine) and 5-HT_{1A} receptors which might account for the effects of the subchronic treatment. However, fluoxetine and norfluoxetine have negligible affinity for 5-HT_{1A} receptors (Hyttel, 1994) so a direct interaction between 5-HT_{1A} receptors and the SSRI or its metabolite is unlikely to account for the current findings.

Female rat sexual behavior includes receptivity, measured with the L/M ratio, lordosis quality and proceptivity (measured by number of hops and darts). L/M ratios have been reported to be the most sensitive indicator of acute hypothalamic 5-HT_{1A} receptor activation (Uphouse, 2000). This was also true for fluoxetine since lordosis quality was only modestly reduced by even the highest dose of fluoxetine (15 mg/kg) examined. Hopping and darting behavior, which is seldom present in the absence of progesterone (Blaustein, 2008; Erskine, 1989), was reduced by acute fluoxetine treatment in the first experiment, but this was not apparent in the third experiment, partly due to a relatively low incidence of proceptivity in the pretest. Thus, fluoxetine's potential effect on proceptivity remains unclear.

Although these findings are each consistent with the hypothesis that enhanced activation of 5-HT_{1A} receptors contributes to the effects of fluoxetine on female sexual behavior, it is important to note that lordosis behavior and human sexual behavior are considerably different. Nevertheless, effects of pharmacological agents on lordosis behavior may still be a good predictor of their effects in the human population in much the same way that animal models of depression have been useful for examination of antidepressant drugs (Cryan et al., 2002; Lapid-Bluhm et al., 2008; Lucki, 1997). However, if 5-HT_{1A} receptors contribute to human, as well as rat, sexual dysfunction following fluoxetine treatment, then the fluoxetine-induced desensitization of 5-HT_{1A} receptors that occurs after chronic treatment would be expected to lead to improvement from sexual dysfunction. In most reports from the human literature, there is the implication that fluoxetine-induced sexual dysfunction fails to improve with continued treatment (Gregorian et al., 2002; Taylor, 2006). However, in many such reports, there has been only limited emphasis on the contribution of the depression, itself, to the sexual dysfunction. When this confounding variable is removed, some improvement may be present (Michelson et al., 2001; Piazza et al., 1997). However, affected women may discontinue treatment prior to reaching this stage of improvement. It is also important to note that the current studies were conducted in ovariectomized rats, with exogenous hormonal priming. In intact Fischer female rats, daily treatment with 10 mg/kg fluoxetine rapidly disrupted estrous cyclicity and lordosis inhibition occurred coincident with the cycle disruption (Uphouse et al., 2006). Improvement in sexual behavior only occurred following 15 to 20 days of fluoxetine treatment, later than the subchronic condition of the current study. ^a

In summary, the present findings implicate 5-HT_{1A} receptors in fluoxetine-induced sexual dysfunction. However, additional studies are needed in both animal models and in the human population before definite conclusions about the brain regions involved and mechanisms responsible for SSRI-induced sexual dysfunction can be clarified.

Acknowledgments

Research supported by NIH HD28419, by TWU REP, and by the Department of Biology at TWU. We thank Ms. Karolina Blaha-Black and Mr. Dan Wall for animal care. The technical assistance of Mr. James Hassell is gratefully acknowledged.

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TIME LINE FOR EXPERIMENT 1

Figure 1. Time line for the protocol for experiment 1

The figure indicates the events that occurred during the 19 days of experimental treatment. A dose of 10 μ g estradiol benzoate (EB) was injected on Day 8 and 500 μ g progesterone (P) was given on Day 10. Four to six hr later, the experiment was initiated with a sexual behavior pretest followed immediately by injection with 1 mg/kg WAY100635 (WAY) or saline. Fifteen min later, rats were injected with 15 mg/kg fluoxetine (FLX); 30 min later, females were returned to the male's cage for sexual behavior testing.

Figure 2. The effect of WAY100635 on fluoxetine-induced lordosis inhibition

Hormonally primed, OVX female rats were pretreated with 1 mg/kg WAY100635 or saline 15 min before injection with 15 mg/kg fluoxetine (N = 10 per treatment). The mean $\pm$ SE L/M ratios for the pretest (PRE) and three consecutive 5-min intervals beginning 30 min after injection with fluoxetine are shown. The absence of visible SE bars indicates the absence of variability or an SE smaller than the symbol. # indicates time points where L/M ratios of rats pretreated with WAY100635 differed significantly from rats pretreated with saline. * indicates time points where the L/M ratio differed significantly from the pretest.

Figure 3. Prior fluoxetine treatment reduced the effect of 8-OH-DPAT on L/M ratios

Hormonally primed, OVX female rats were pretreated with fluoxetine (F) or distilled water (W) for 9 days before injection with 8-OH-DPAT (DPAT). The mean $\pm$ SE L/M ratios for the pretest (PRE) and six consecutive 5-min intervals after injection with 0 and 25 μ g/kg 8-OH-DPAT ($N = 6$ per treatment) are shown in A; with 50 and 75 μ g/kg 8-OH-DPAT (for 50 and 75 μ g 8-OH-DPAT; $N = 7$ and 6 for fluoxetine and 8 and 6 for water) in B; and with 100 μ g/kg 8-OH-DPAT ($N = 4$ per treatment) in C. The absence of visible SE bars indicates the absence of variability or an SE smaller than the symbol. # indicates the time points where L/M ratios of rats treated with 8-OH-DPAT were significantly different from rats treated with 0 μ g/kg 8-OH-DPAT within pretreatment condition. \$ indicates significant difference within

time and hormone treatment between fluoxetine and water pretreatments. * indicates time points where the L/M ratios were first significantly different from the pretest and remained significantly differed at every time point thereafter.

Figure 4. Prior fluoxetine treatment, 8-OH-DPAT, and lordosis quality

Data are the mean $\pm$ SE lordosis quality scores of hormonally primed OVX rats treated daily with fluoxetine (FLX) and then injected with varying doses of 8-OH-DPAT. The absence of visible SE bars indicates the absence of variability or an SE smaller than the symbol. The data are for the pretest (PRE) and 5-min intervals after 8-OH-DPAT injection. * indicates time points where the lordosis quality differed significantly from the pretest.

Table 1

Effect of fluoxetine on L/M ratios in EO and EP rats

OVX rats were hormonally primed with estradiol benzoate and oil (EO) or estradiol benzoate and progesterone (EP) and injected with varying doses of fluoxetine (FLX). Shown in the table are the mean $\pm$ SE L/M ratios for the pretest and three consecutive 5-min intervals beginning 30 min after fluoxetine injection.

Fluoxetine treatment	Hormone treatment	Mean $\pm$ S.E. L/M Ratios			
		Pretest	5 min	10 min	15 min
0 mg/kg	EO (n = 5)	0.94 $\pm$ 0.04	0.95 $\pm$ 0.05	0.95 $\pm$ 0.05	0.88 $\pm$ 0.1
	EP (n = 4)	0.97 $\pm$ 0.03	0.92 $\pm$ 0.03	1 $\pm$ 0	1 $\pm$ 0
2.5 mg/kg	EO (n = 6)	0.88 $\pm$ 0.05	0.83 $\pm$ 0.12	0.85 $\pm$ 0.12	0.91 $\pm$ 0.08
	EP (n = 6)	0.98 $\pm$ 0.02	1 $\pm$ 0	1 $\pm$ 0	1 $\pm$ 0
5 mg/kg	EO (n = 6)	0.85 $\pm$ 0.05	0.65 $\pm$ 0.11	0.51 $\pm$ 0.17 ^{§##}	0.49 $\pm$ 0.11 ^{§##}
	EP (n = 6)	0.98 $\pm$ 0.02	0.74 $\pm$ 0.14	0.74 $\pm$ 0.14	0.85 $\pm$ 0.11
7.5 mg/kg	EO (n = 6)	0.93 $\pm$ 0.03	0.48 $\pm$ 0.16 ^{§##}	0.62 $\pm$ 0.11 ^{§*}	0.66 $\pm$ 0.09 [§]
	EP (n = 6)	1 $\pm$ 0	0.73 $\pm$ 0.18	0.87 $\pm$ 0.08	0.94 $\pm$ 0.04
10 mg/kg	EO (n = 8)	0.92 $\pm$ 0.04	0.44 $\pm$ 0.12 ^{##}	0.41 $\pm$ 0.16 ^{§##}	0.51 $\pm$ 0.12 ^{##}
	EP (n = 8)	1 $\pm$ 0	0.48 $\pm$ 0.11 ^{##}	0.69 $\pm$ 0.12	0.59 $\pm$ 0.14 ^{##}
15 mg/kg	EO (n = 6)	0.87 $\pm$ 0.04	0.18 $\pm$ 0.12 ^{##}	0.06 $\pm$ 0.06 ^{##}	0.31 $\pm$ 0.16 ^{##}
	EP (n = 6)	1 $\pm$ 0	0.08 $\pm$ 0.08 ^{##}	0.30 $\pm$ 0.16 ^{##}	0.31 $\pm$ 0.17 ^{##}

indicates the time points where L/M ratios of rats for the same hormone treatment were significantly different from rats treated with 0 mg/kg fluoxetine.

§ indicates time points where L/M ratios of EO rats were significantly different from those of the same dose EP rats.

* indicates time points where the L/M ratio differed significantly from the pretest.