Published in final edited form as:

Pharmacol Biochem Behav. 2013 December ; 0: 52–57. doi:10.1016/j.pbb.2013.10.024.

Comparison of Female Fischer and Sprague-Dawley Rats in the Response to Ketanserin

Chandra Suma Johnson Miryala, Cindy Hiegel, and Lynda Uphouse

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

Abstract

The effects of the 5-HT_{2A/2C} receptor antagonist, ketanserin, on lordosis behavior were examined in hormonally primed, ovariectomized Fischer and Sprague-Dawley females. Rats were primed with 0.067 µg/g body weight estradiol benzoate and 3.33 µg/g body weight progesterone. After a pretest for sexual behavior, rats were injected with 0.416 to 10 mg/kg ketanserin. In both strains, lordosis behavior, lordosis quality, and proceptivity were significantly reduced by ketanserin. There was modest evidence of a strain difference with Sprague-Dawley females slightly more sensitive to ketanserin. In a second experiment, the effects of 10 mg/kg fluoxetine, 1 mg/kg ketanserin, and their combination were examined to determine if the two drugs would have additive effects on sexual behavior. There was no evidence that the drugs were additive in their effect and the strains did not differ in their response to the combined treatment. These findings are discussed in relation to prior evidence for strain differences in the sexual behavioral response to fluoxetine and to a receptor agonist acting preferentially at 5-HT_{1A} receptors.

Keywords

Rat strains; ovariectomized; fluoxetine; 5-HT₂ receptors; lordosis behavior; proceptivity

1.0 Introduction

A role for serotonin (5-HT) in the modulation of female rat sexual behavior is widely recognized (Mendelson and Gorzalka, 1990; Uphouse and Guptarak, 2010). A variety of drugs that increase extracellular 5-HT inhibit lordosis behavior but, depending on the receptor subtype activated, 5-HT receptor agonists can either inhibit or facilitate the behavior (Gonzalez et al., 1997; Hunter et al., 1985; Uphouse et al., 1996; Uphouse and Caldarola-Pastuszka, 1993; Wolf et al., 1998). The best characterized such agonists are the 5-HT_{1A} receptor agonists which rapidly inhibit lordosis behavior (Mendelson, 1992; Uphouse, 2000). As a result, it has been generally assumed that increased extracellular 5-HT reduces lordosis behavior by activation of 5-HT_{1A} receptors. In contrast, agonists that act primarily on 5-HT₂ or 5-HT₃ receptors facilitate lordosis behavior in female rats with relatively low sexual receptivity (Mendelson and Gorzalka, 1985; Wolf et al., 1999; Wolf et

© 2013 Elsevier Inc. All rights reserved.

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; Ms. Chandra Suma Johnson Miryala, Department of Biology, Texas Woman's University, Denton, Texas 76204, Phone: 940-898-2369, Fax: 940-898-2382, CMiryala@twu.edu; Ms. Cindy Hiegel, Department of Biology, Texas Woman's University, Denton, Texas 76204, Phone: 940-898-2389, Fax: 940-898-2382, CHiegel@twu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

al., 1998). A potentially beneficial effect of 5-HT₂ and 5-HT₃ receptors is inferred from observations that 5-HT₂ and 5-HT₃ receptor agonists protect against the lordosis-inhibiting effects of 5-HT_{1A} receptor agonists (Maswood et al., 1998; Uphouse et al., 1994) and that 5-HT₂ and 5-HT₃ receptor antagonists inhibit lordosis behavior (Gonzalez et al., 1997; Maswood et al., 1997). Therefore, drugs which lead to generalized increases in extracellular 5-HT could disrupt the balance between activation of 5-HT receptors that inhibit and those that facilitate lordosis behavior. The relevance of such a disruption is evidenced by the large number of human females who show sexual dysfunction following treatment with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Clayton et al., 2006; Clayton, 2002; Gelenberg et al., 2000; Gregorian et al., 2002).

SSRIs block the serotonin transporter (SERT) and thereby lead to an increase in extracellular 5-HT and enhanced activation of all 5-HT receptors (Fuller et al., 1991; Gobert et al., 1997; Perry and Fuller, 1992, 1993; Sghendo and Mifsud, 2011; Tao et al., 2002; Tavoulari et al., 2009), but it is the activation of 5-HT_{1A} receptors that has been postulated to account for the lordosis inhibition that follows treatment with fluoxetine (Guptarak et al., 2010). However, not all rat strains show comparable vulnerability to the lordosis-inhibiting effects of either fluoxetine or a 5-HT_{1A} receptor agonist (Miryala et al., 2013; Uphouse et al., 2002). For example, Fischer rats have a higher baseline level of 5-HT than Sprague-Dawley rats (Rosecrans et al., 1986), show an accentuated 5-HT response to stress (Dhabhar et al., 1993; Kosten and Ambrosio, 2002; Rosecrans et al., 1986), and are more responsive to the lordosis-inhibiting effects of fluoxetine than are Sprague-Dawley females (Maswood et al., 2008; Miryala et al., 2013; Uphouse et al., 2006). However, Fischer females are less responsive than Sprague-Dawley females to the lordosis-inhibiting effects of a 5-HT_{1A} receptor agonist (Uphouse et al., 2002) suggesting that strain differences in the lordosis response to fluoxetine may include additional 5-HT receptors. To date, there have been no studies of a potential rat strain difference in the response to either 5-HT₂ or 5-HT₃ receptor active compounds. Therefore, in the following experiment, a potential strain difference in the sexual behavioral response to the 5-HT_{2A/2C} receptor antagonist, ketanserin, was examined. Emphasis was placed on the 5-HT_{2A/2C} receptor because this receptor has been most thoroughly investigated for its impact on the response to 5-HT_{1A} receptor agonists (Uphouse and Guptarak, 2010) and because such antagonists can augment the effects of SSRIs (Boothman et al., 2006; Cremers et al., 2004; Marek et al., 2003; Marek et al., 2005). Such augmentation is thought to result from the ability of 5-HT_{2A/2C} receptor antagonists to increase firing of 5-HT neurons in the dorsal raphe nucleus (DRN) (Boothman et al., 2003; Boothman and Sharp, 2005; Cremers et al., 2004). Therefore, 5-HT_{2A/2C} receptor antagonists, by increasing extracellular 5-HT in lordosis-controlling brain areas, would be expected to reduce lordosis behavior both by increasing activation of inhibitory 5-HT_{1A} receptors and by antagonizing any protective effect of 5-HT_{2A/2C} receptors. Because of the greater sensitivity of Sprague-Dawley rats to the lordosis-inhibiting effect of a 5-HT_{1A} receptor agonist but lesser sensitivity to the SSRI, fluoxetine, it was hypothesized that Sprague-Dawley females might show a greater sensitivity to antagonism of 5-HT_{2A/2C} receptors.

2.0 Materials and Methods

2.1 Materials

Estradiol benzoate (EB), progesterone (P), sesame seed oil, the selective serotonin reuptake inhibitor (SSRI), fluoxetine (methyl[3-phenyl-3-4-(trifluoromethyl)-phenoxy] propyl] ammonium chloride), and the 5-HT_{2A/2C} receptor antagonist, ketanserin (3-[2-[4-(4-fluorbenzoyl)-1-piperidinyl]ethyl]- 2,4(1H,3H)-quinazolinedione), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Isoflurane (AErrane®) and suture materials were purchased from Butler Schein Animal Health (Dublin, OH). Food (Rodent

Lab Diet 5001) was purchased from Lab Animal Supply (Highland Village, TX, USA). All other supplies were purchased from Fisher Scientific (Houston, TX, USA).

2.2 Methods

All procedures were in accordance with the NIH Guide for the Care and Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Texas Woman's University.

2.2.1 Animals and Housing Conditions—Female Fischer and Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2 per cage in standard shoebox caging ($45.72 \times 24.13 \times 20.59$ cm). The housing area was maintained at 25° C and 65% humidity with lights on from 12:00 midnight to 12:00 noon. Animals varied in their age within experiments but were always matched between Fischer and Sprague-Dawley females and counterbalanced between treatment conditions. Food and water were available *ad libitum*.

2.2.2 Surgical Procedures—Rats were ovariectomized under AErrane® anaesthesia as described previously (White and Uphouse, 2004) approximately 2 weeks after their arrival. Hormonal priming was with EB followed 48 hours later with P and occurred 10 to 14 days after ovariectomy. Hormones were dissolved in sesame seed oil and injected subcutaneously. Since Fischer and Sprague-Dawley females differ in their body weight, hormones were administered per body weight (0.067 µg/g EB and 3.333 µg/g P). At the time of behavioral testing, in the first experiment, females were 12 to 22 weeks old; the mean $\pm$ standard error (S.E.) body weights of Fischer and Sprague-Dawley were 162.7 ± 1.55 and 273.0 ± 4.15 , respectively; in the second experiment, females were 13 to 17 weeks old with mean $\pm$ S.E. body weights of Fischer and Sprague-Dawley rats of 166.6 ± 2.05 and 264.2 ± 3.85 , respectively.

2.2.3 Treatment of Animals—On the day of testing, 4 to 6 hr after P injection, rats were pretested for sexual behavior as previously described (White and Uphouse, 2004). Rats were placed into the home cage of a sexually active male and behavior was monitored until 10 mounts had occurred or for a maximum of 10 min. In the first experiment, immediately following the pretest, females were injected intraperitoneally (ip) with deionized water (DI) or with 0.416, 0.5, 0.75, 1.0, 2.0, 5.0 or 10.0 mg/kg of the 5-HT_{2A/2C} receptor antagonist, ketanserin, in a volume of 1 ml/kg. Behavioral testing began immediately after the ketanserin injection and was recorded for 30 consecutive min. Data were grouped into the first and second 15 min intervals for statistical analysis. In the second experiment, immediately following the pretest, females were injected with fluoxetine (10 mg/kg; volume of 1 ml/kg) or deionized water (DI). Fifteen min later, DI treated rats were injected intraperitoneally with 1 mg/kg ketanserin; fluoxetine-treated rats were injected with ketanserin (1 mg/kg) or DI. This led to three groups: fluoxetine only, ketanserin only, and the combination of fluoxetine and ketanserin. Fifteen min after the ketanserin or DI treatment, females were placed into the male's cage for 15 min of sexual behavioral testing. Data for the 15 min interval were grouped for statistical comparison.

2.2.4 Behavioral Testing Procedures—The lordosis/mount (L/M) ratio (number of lordosis reflexes by the female divided by the number of mounts by the male) and lordosis quality scores (on a scale of 1 to 4) were measured as previously described (White and Uphouse, 2004). Lordosis quality was calculated as the sum of the individual lordosis quality scores divided by the number of lordosis responses. Proceptivity was measured by the presence or absence of hopping and darting behavior. L/M ratios, lordosis quality scores,

and mounts were recorded for the pretest and for the 15-min test intervals. Proceptive behavior was recorded as present or absent for the pretest and the entire test interval.

2.2.5 Statistical Procedures—For the first experiment, L/M ratios, lordosis quality and number of mounts were analyzed by repeated measures analysis of variance (ANOVA) with strain and dose of ketanserin as independent factors and with first or second testing interval as the repeated factor. L/M ratios were subjected to regression on dose for estimation of the IC_{50} . IC_{50} (dose leading to 50% of maximum effect) was defined as an L/M ratio of 0.5. For experiment 2, strain and treatment condition were independent factors and time relative to pretest was the repeated factor. Proceptivity data were analyzed using Chi-Square and Fischer's Exact Test procedures. Data were analyzed with SPSS version 19.0 (for PC) and the statistical reference was Zar (Zar, 2010). Post-hoc Tukey comparisons were computed manually and an alpha of 0.05 was required for statistical significance.

3.0 Results

The effects of ketanserin on lordosis behavior of Fischer and Sprague-Dawley females are shown in Figure 1. There was a significant main effect of strain ($F_{1,109} = 5.14$, $p = 0.025$) and dose of ketanserin ($F_{7,109} = 11.47$, $p = 0.001$) but not a significant interaction between strain and dose ($p > 0.05$). The strain difference reflected a lower L/M ratio of Sprague-Dawley than Fischer females that was most evident during the second 15 min interval after ketanserin treatment. In comparison to the DI water control, Sprague-Dawley females showed a significant decline in the L/M ratio at lower doses of ketanserin than were evident in Fischer females. However, the IC_{50} for the two strains were relatively similar. For the first 15 min interval, the IC_{50} for Fischer and Sprague-Dawley, respectively, was 12.7 and 10.6 mg/kg. For the second 15 min interval, the IC_{50} for Fischer and Sprague-Dawley, respectively, were 6.12 and 7.5 mg/kg. There was a significant difference between the first and second 15 min interval (ANOVA for time after ketanserin, $F_{1,109} = 39.93$, $p = 0.001$), but there was no interaction between time and strain ($p > 0.05$) and none of the interactions with strain were significant (all $p > 0.05$).

The effects of ketanserin on lordosis quality were similar to those for L/M ratios and were also more obvious during the second 15 min interval (ANOVA for time, $F_{1,106} = 9.27$, $p = 0.003$, see Figure 2). There was a significant effect of the dose of ketanserin on lordosis quality ($F_{7,106} = 4.14$, $p = 0.001$) and, as for L/M ratios, quality scores were slightly more likely to be affected in Sprague-Dawley than in Fischer females (ANOVA for strain, $F_{1,106} = 9.21$, $p = 0.003$). Nevertheless, none of the interactions with strain or dose were significant (all $p > 0.05$).

Fischer and Sprague-Dawley females received comparable mounting from the males. The only significant effect was between strain and time ($F_{1,110} = 5.72$, $p = 0.018$) that reflected a decline between the first and second 15 min intervals in number of mounts for Fischer, but not Sprague-Dawley rats. For Fischer rats, the means $\pm$ S.E. number of mounts for the 15 and 30 min intervals, respectively, were 20.5 ± 0.97 and 17.8 ± 0.98 ; for Sprague-Dawley females, the means $\pm$ S.E. were 19.2 ± 0.98 and 19.8 ± 0.99 , respectively.

Prior to injection, a greater proportion of Sprague-Dawley than Fischer females showed evidence of proceptivity (for Fischer and Sprague-Dawley, respectively, 61.9% and 80.9%; Fisher Exact test, $p = 0.029$). After injection, there was a significant effect of the treatment in Fischer (Chi-Square = 20.35, $df = 7$, $p = 0.005$) but this reflected a decrease in proceptivity relative to the DI control. When only ketanserin groups were compared, there was no dose effect ($p > 0.05$). There was not a significant effect of treatment on proceptivity of Sprague-Dawley females when individual doses were examined (Chi-Square = 9.3, $df =$

7, $p > 0.05$) (Figure 3). However, when groups were collapsed across the dose of ketanserin and compared to the DI control, proceptivity of ketanserin-treated females was significantly reduced in both strains (see Figure 3 inset; for Fischer and Sprague-Dawley, respectively, Chi-Square = 13.08, $p = 0.001$ and Chi-Square = 4.93, $p = 0.026$).

The second experiment was designed to determine if fluoxetine and ketanserin would amplify the effects of either drug alone. A dose of 10 mg/kg fluoxetine (Miryala et al., 2013) and 1 mg/kg ketanserin (Experiment 1) were chosen and the effects of either drug or their combination were compared. For L/M ratios, there was a significant effect of the type of treatment ($F_{2, 45} = 4.43$, $p = 0.017$), time ($F_{1, 45} = 90.71$, $p = 0.0001$) and their interaction ($F_{2, 45} = 3.39$, $p = 0.042$) (Figure 4). Neither strain nor any interactions with strain were significant. All treatment conditions reduced L/M ratios relative to their pretest ($q_{45, 2} = 2.85$, $p < 0.05$) but L/M ratios after fluoxetine injection were higher than in the other 2 groups. This was especially evident in Fischer female where fluoxetine-treated rats had significantly higher L/M ratios than the other two treatment conditions (for ketanserin, alone, and both drugs, respectively, $q_{3, 45} = 3.65$ and 5.25 , $p = 0.05$). For Sprague-Dawley females, L/M ratios of fluoxetine-treated rats did not differ significantly from either of the 2 other groups (Tukey's $q_{3, 45}$, all $p > 0.05$). In neither strain did the L/M ratios of rats treated with both ketanserin and fluoxetine differ from L/M ratios after ketanserin, alone (all $p > 0.05$).

Two females (one from each strain) failed to show any lordosis behavior after treatment and were excluded from the analysis for lordosis quality. For the remaining rats, there was not a differential effect of treatment on lordosis quality ($p > 0.05$; see Table 1). Although there was a slight effect of strain ($F_{1, 43} = 5.50$, $p = 0.05$) and time ($F_{1, 43} = 18.42$, $p = 0.001$), lordosis quality remained relatively high throughout the testing period with the exception of Sprague-Dawley female given both drugs. For this treatment, in this strain, lordosis quality was significantly reduced relative to the pretest ($q_{3, 43} = 5.93$, $p = 0.05$) and the combination of the two drugs differed from both fluoxetine only and ketanserin only groups (respectively, $q_{3, 43} = 5.07$ and 5.33 , $p = 0.05$).

As in the first experiment, more Sprague-Dawley than Fischer females showed proceptivity (34.6% of Fischer and 92% of Sprague-Dawley females were proceptive; Chi-Square = 17.95, $df = 1$, $p = 0.001$). Rats assigned to different treatment conditions did not differ in the pretest (Chi-Square for Fischer = 0.552 and Sprague-Dawley = 1.0, $p > 0.05$) but there was a treatment-dependent effect on proceptivity after treatment (respectively for Fischer and Sprague-Dawley females, Chi-Square = 7.63 and 7.29, $df = 2$, $p = 0.03$) (Figure 5). For Sprague-Dawley females, the treatment effect resulted from higher proceptivity in fluoxetine-treated rats compared to the other 2 groups (for ketanserin plus fluoxetine and ketanserin, alone, respectively, Chi-Square = 6.34 and 4.96, $df = 1$, $p = 0.04$). Although a similar pattern was present in Fischer females, because of the low overall proceptivity in Fischer rats, there were no treatment effects. Strain differences were present only in rats given fluoxetine (Chi-Square = 5.52, $df = 1$, $p = 0.04$) and reflected the lower overall proceptivity of Fischer females rather than a strain difference in response to the treatment.

There were no differences between strains or among treatments on the number of mounts received by the females. The mean $\pm$ S.E. number of mounts for Fischer rats for fluoxetine only, ketanserin only, and their combination, respectively, were 21.7 ± 2.6 , 15.42 ± 2.31 and 14.7 ± 1.84 ; the average number of mounts for fluoxetine only, ketanserin only and their combination for Sprague-Dawley, respectively, were 14.4 ± 2.48 , 16.7 ± 2.0 , and 13.1 ± 1.76 .

4.0 Discussion

The present study was designed to expand information about the strain differences in the 5-HT system which could potentially account for the lower sensitivity of Sprague-Dawley females to the lordosis inhibiting effects of fluoxetine but greater sensitivity to the lordosis-inhibiting 5-HT_{1A} receptor agonist, (+/-) 8-hydroxy-2- (di-n-propylamino) tetralin (8-OH-DPAT). Ketanserin reduced lordosis behavior in both strains and inhibition was greatest during the second 15 min test interval (15 to 30 min after injection), consistent with earlier studies showing time-dependent effects of ketanserin following infusion into the ventromedial nucleus of the hypothalamus (Sinclair-Worley and Uphouse, 2004; Wolf et al., 1998). In comparison to robust strain differences in the lordosis-inhibitory effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Uphouse et al., 2002), strain differences in the response to the 5-HT_{2A/2C} receptor antagonist were relatively modest and evident only during the 2nd 15 min test interval. Nevertheless, the IC₅₀ was comparable in the two strains. However, that Sprague-Dawley females showed a slightly greater effect of ketanserin may indicate a greater dependency of this strain on the lordosis-facilitating effects of 5-HT_{2A/2C} receptors.

Ketanserin's ability to reduce lordosis behavior is believed to result from its antagonism of 5-HT_{2A/2C} receptors (Mendelson, 1992; Uphouse and Guptarak, 2010) but whether 5-HT_{2A} or 5-HT_{2C} receptors are responsible is unclear. Ketanserin blocks both 5-HT_{2A} and 5-HT_{2C} receptors but is reported to have the greater affinity for 5-HT_{2A} receptors (Hoyer et al., 2002). However, in the VMN, it is 5-HT_{2C} receptors that have been implicated in lordosis modulation (Wolf et al., 1999). In this brain area, the 5-HT_{2B/2C} receptor antagonist, SB206553, but not the 5-HT_{2A} receptor antagonist, MDL 100,907, mimicked effects of ketanserin. In contrast, 5-HT_{2A} receptors may be responsible for 5-HT₂ receptor-mediated drug effects in the medial preoptic area (Gonzalez et al., 1997). Therefore, both 5-HT_{2A} and 5-HT_{2C} receptors and several brain areas may contribute to the decline in lordosis behavior after the systemic treatment with ketanserin.

Differences between Fischer and Sprague-Dawley rats have been repeatedly noted with considerable evidence for differential functioning of the 5-HT system (Burnet et al., 1994; Fernandez et al., 2003; Miryala et al., 2013; Rosecrans et al., 1986; Uphouse et al., 2002). Given the inhibitory effects of 5-HT on female sexual behavior (Uphouse and Guptarak, 2010), this strain difference in 5-HT functioning may contribute to baseline strain differences in sexual behavior such as proceptivity as previously reported (Miryala et al., 2013) and as observed in the current study. It is worth noting that the effect of fluoxetine on L/M ratios in the two strains was comparable to that reported in our prior study while effects of fluoxetine on proceptivity were not. In our previous report, 10 mg/kg fluoxetine significantly reduced proceptivity in Sprague-Dawley females while no such decline was evident in the current study. We do not have a ready explanation for the difference in the two reports. Since only a single dose of fluoxetine was used in the current study, greater effects on proceptivity may have been evident had a higher dose been used.

However, of special relevance to the current study is evidence that Fischer rats may have more SERT in the DRN than other rat strains (Burnet et al., 1996; Fernandez et al., 2003). If so, relative to Sprague-Dawley females, Fischer rats would be expected to have less extracellular 5-HT in the DRN following treatment with the SERT blocker, fluoxetine. With less consequent activation of the somatodendritic autoinhibitory 5-HT_{1A} receptors, Fischer females might show a greater elevation of extracellular 5-HT in lordosis-relevant brain areas such as the VMN following treatment with SSRIs. With fluoxetine's additional antagonism at 5-HT₂ receptors (Jenck et al., 1993; Palvimäki et al., 1996), the increase in 5-HT release

would disrupt the balance between the lordosis-inhibiting (5-HT_{1A}) and lordosis-facilitating (5-HT_{2C}) receptors to a greater extent in Fischer than in Sprague-Dawley females.

In contrast, following treatment with a 5-HT_{1A} receptor agonist, the higher midbrain SERT of Fischer females would not impact the strain response since the agonist would directly activate lordosis-inhibiting 5-HT_{1A} receptors to reveal a greater sensitivity of Sprague-Dawley females to the 5-HT_{1A} receptor agonist.

The current studies offer indirect evidence that strain differences between Fischer and Sprague-Dawley females do not include 5-HT₂ receptors. 5-HT₂ receptors in the midbrain decrease firing of DRN neurons possibility by activation of inhibitory GABA neurons (Boothman et al., 2003; Boothman and Sharp, 2005) and 5-HT₂ receptor antagonists increase release of 5-HT in areas terminal to DRN neurons (Boothman et al., 2006). In the current study, blocking 5-HT₂ receptors with ketanserin would be expected to increase DRN firing and release of 5-HT and thereby increase activation of 5-HT_{1A} receptors and reduce activation of 5-HT₂ receptors in lordosis-relevant brain areas. It was, therefore, expected that Sprague-Dawley rats would show greater sensitivity to ketanserin, but this was not the case. In the absence of a remarkable strain difference in the response to 5-HT₂ receptor antagonism, the resulting disruption of balance between the inhibitory and facilitatory 5-HT₂ receptors would be substantial in both strains but would produce a minimal strain difference, as observed in the current study.

Since fluoxetine not only blocks SERT but also blocks 5-HT₂ receptors (Jenck et al., 1993; Palvimäki et al., 1996), it was anticipated that ketanserin plus fluoxetine would be additive in their effect on lordosis behavior. Such an accentuated response in hippocampal extracellular 5-HT has been reported in Fischer females following treatment with another SSRI, citalopram, but not following fluoxetine (Fernandez et al., 2003). However, such an augmentation following fluoxetine has been reported by other investigators (Cremers et al., 2004). Nevertheless, in the current study, fluoxetine and ketanserin were not additive in their effect on lordosis behavior and there was no evidence of a strain difference.

Therefore, the current studies provide evidence that previously reported difference between Fischer and Sprague-Dawley females in response to fluoxetine or a 5-HT_{1A} receptor agonist are unlikely to depend on a strain difference in 5-HT₂ receptor action. However, since ketanserin blocks both 5-HT_{2A} and 5-HT_{2C} receptors, more selective 5-HT₂ receptor antagonists are needed to definitively eliminate 5-HT₂ receptors from consideration. Moreover, only a single dose of fluoxetine and ketanserin were examined in the current study. Additional dose combinations would be useful to completely rule out such a strain difference in 5-HT₂ receptors and/or additive/synergistic effects of 5-HT₂ receptor antagonists and fluoxetine. Finally, it is important to note that ketanserin also blocks alpha-1 adrenergic receptors (Hoyer et al., 1987; Marwood, 1994) so that we cannot rule out possible strain differences in these receptors that could have masked the existence of a strain difference for 5-HT₂ receptor antagonism.

In summary, ketanserin dose-dependently inhibited lordosis behavior in both Fischer and Sprague-Dawley females. Strain differences, though present, were modest and are more likely to reflect the greater sensitivity of Sprague-Dawley females to 5-HT_{1A} receptor activation than a strain difference in 5-HT₂ receptor functioning. In addition, ketanserin and fluoxetine were not additive in their effect on lordosis, and the combined treatment reflected primarily the lordosis inhibition with ketanserin alone.

Acknowledgments

Research supported by NIH HD28419 and by a TWU institutional research support grant. Special appreciation is given to Ms. Aminata Diaby for technical assistance. We thank Ms. Karolina Blaha-Black and Mr. Dan Wall for animal care.

References

- Boothman LJ, Allers KA, Rasmussen K, Sharp T. Evidence that central 5-HT_{2A} and 5-HT_{2B/C} receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. *Br J Pharmacol*. 2003; 139:998–1004. [PubMed: 12839874]
- Boothman LJ, Mitchell SN, Sharp T. Investigation of the SSRI augmentation properties of 5-HT(2) receptor antagonists using in vivo microdialysis. *Neuropharmacology*. 2006; 50:726–732. [PubMed: 16434063]
- Boothman LJ, Sharp T. A role for midbrain raphe gamma aminobutyric acid neurons in 5-hydroxytryptamine feedback control. *Neuroreport*. 2005; 16:891–896. [PubMed: 15931057]
- Burnet PW, Mefford IN, Smith CC, Gold PW, Sternberg EM. Hippocampal 5-HT_{1A} receptor binding site densities, 5-HT_{1A} receptor messenger ribonucleic acid abundance and serotonin levels parallel the activity of the hypothalamo-pituitary-adrenal axis in rats. *Behav Brain Res*. 1996; 73:365–368. [PubMed: 8788533]
- Burnet PW, Michelson D, Smith MA, Gold PW, Sternberg EM. The effect of chronic imipramine administration on the densities of 5-HT_{1A} and 5-HT₂ receptors and the abundances of 5-HT receptor and transporter mRNA in the cortex, hippocampus and dorsal raphe of three strains of rat. *Brain Res*. 1994; 638:311–324. [PubMed: 8199869]
- Clayton A, Keller A, McGarvey EL. Burden of phase-specific sexual dysfunction with SSRIs. *J Affect Disord*. 2006; 91:27–32. [PubMed: 16430968]
- Clayton AH. Female sexual dysfunction related to depression and antidepressant medications. *Curr Womens Health Rep*. 2002; 2:182–187. [PubMed: 12099193]
- Cremers TI, Giorgetti M, Bosker FJ, Hogg S, Arnt J, Mork A, Honig G, Bogeso KP, Westerink BH, den Boer H, Wikstrom HV, Tecott LH. Inactivation of 5-HT(2C) receptors potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology*. 2004; 29:1782–1789. [PubMed: 15138437]
- Dhabhar FS, McEwen BS, Spencer RL. Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels--a comparison between Sprague-Dawley, Fischer 344 and Lewis rats. *Brain Res*. 1993; 616:89–98. [PubMed: 8395308]
- Fernandez F, Sarre S, Launay JM, Aguerre S, Guyonnet-Duperat V, Moisan MP, Ebinger G, Michotte Y, Mormede P, Chaouloff F. Rat strain differences in peripheral and central serotonin transporter protein expression and function. *Eur J Neurosci*. 2003; 17:494–506. [PubMed: 12581167]
- Fuller RW, Wong DT, Robertson DW. Fluoxetine, a selective inhibitor of serotonin uptake. *Med Res Rev*. 1991; 11:17–34. [PubMed: 1994152]
- Gelenberg AJ, Delgado P, Nurnberg HG. Sexual side effects of antidepressant drugs. *Curr Psychiatry Rep*. 2000; 2:223–227. [PubMed: 11122960]
- Gobert A, Rivet JM, Cistarelli L, Millan MJ. Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY 100,635 and GR 127,935. *J Neurochem*. 1997; 68:1159–1163. [PubMed: 9048762]
- Gonzalez MI, Greengrass P, Russell M, Wilson CA. Comparison of serotonin receptor numbers and activity in specific hypothalamic areas of sexually active and inactive female rats. *Neuroendocrinology*. 1997; 66:384–392. [PubMed: 9430444]
- Gregorian RS, Golden KA, Bahce A, Goodman C, Kwong WJ, Khan ZM. Antidepressant-induced sexual dysfunction. *Ann Pharmacother*. 2002; 36:1577–1589. [PubMed: 12243609]
- Guptarak J, Sarkar J, Hiegel C, Uphouse L. Role of 5-HT(1A) receptors in fluoxetine-induced lordosis inhibition. *Horm Behav*. 2010; 58:290–296. [PubMed: 20223238]
- Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav*. 2002; 71:533–554. [PubMed: 11888546]

- Hoyer D, Vos P, Closse A, Pazos A, Palacios JM, Davies H. [3H]ketanserin labels 5-HT₂ receptors and alpha 1-adrenoceptors in human and pig brain membranes. *Naunyn Schmiedebergs Arch Pharmacol.* 1987; 335:226–230. [PubMed: 2884572]
- Hunter AJ, Hole DR, Wilson CA. Studies into the dual effects of serotonergic pharmacological agents on female sexual behaviour in the rat: preliminary evidence that endogenous 5HT is stimulatory. *Pharmacol Biochem Behav.* 1985; 22:5–13. [PubMed: 3156384]
- Jenck F, Moreau JL, Mutel V, Martin JR, Haefely WE. Evidence for a role of 5-HT_{1C} receptors in the antiserotonergic properties of some antidepressant drugs. *Eur J Pharmacol.* 1993; 231:223–229. [PubMed: 8453978]
- Kosten TA, Ambrosio E. HPA axis function and drug addictive behaviors: insights from studies with Lewis and Fischer 344 inbred rats. *Psychoneuroendocrinology.* 2002; 27:35–69. [PubMed: 11750769]
- Marek GJ, Carpenter LL, McDougle CJ, Price LH. Synergistic action of 5-HT_{2A} antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology.* 2003; 28:402–412. [PubMed: 12589395]
- Marek GJ, Martin-Ruiz R, Abo A, Artigas F. The selective 5-HT_{2A} receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology.* 2005; 30:2205–2215. [PubMed: 15886717]
- Marwood JF. Influence of alpha 1-adrenoceptor antagonism of ketanserin on the nature of its 5-HT₂ receptor antagonism. *Clin Exp Pharmacol Physiol.* 1994; 21:955–961. [PubMed: 7736654]
- Maswood N, Caldarola-Pastuszka M, Uphouse L. 5-HT₃ receptors in the ventromedial nucleus of the hypothalamus and female sexual behavior. *Brain Res.* 1997; 769:13–20. [PubMed: 9374268]
- Maswood N, Caldarola-Pastuszka M, Uphouse L. Functional integration among 5-hydroxytryptamine receptor families in the control of female rat sexual behavior. *Brain Res.* 1998; 802:98–103. [PubMed: 9748522]
- Maswood N, Sarkar J, Uphouse L. Modest effects of repeated fluoxetine on estrous cyclicity and sexual behavior in Sprague Dawley female rats. *Brain Res.* 2008; 1245:52–60. [PubMed: 18929547]
- Mendelson SD. A review and reevaluation of the role of serotonin in the modulation of lordosis behavior in the female rat. *Neurosci Biobehav Rev.* 1992; 16:309–350. [PubMed: 1528523]
- Mendelson SD, Gorzalka BB. A facilitatory role for serotonin in the sexual behavior of the female rat. *Pharmacol Biochem Behav.* 1985; 22:1025–1033. [PubMed: 3161094]
- Mendelson SD, Gorzalka BB. Sex differences in the effects of 1-(m-trifluoromethylphenyl) piperazine and 1-(m-chlorophenyl) piperazine on copulatory behavior in the rat. *Neuropharmacology.* 1990; 29:783–786. [PubMed: 2274113]
- Miryala CS, Hiegel C, Uphouse L. Sprague-Dawley and Fischer females differ in acute effects of fluoxetine on sexual behavior. *Journal of Sexual Medicine.* 2013; 10:350–361. [PubMed: 23110651]
- Palvimäki EP, Roth BL, Majasuo H, Laakso A, Kuoppamäki M, Syvälahti E, Hietala J. Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT_{2c} receptor. *Psychopharmacology (Berl).* 1996; 126:234–240. [PubMed: 8876023]
- Perry KW, Fuller RW. Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. *Life Sci.* 1992; 50:1683–1690. [PubMed: 1375306]
- Perry KW, Fuller RW. Extracellular 5-hydroxytryptamine concentration in rat hypothalamus after administration of fluoxetine plus L-5-hydroxytryptophan. *J Pharm Pharmacol.* 1993; 45:759–761. [PubMed: 7901377]
- Rosecrans JA, Robinson SE, Johnson JH, Mokler DJ, Hong JS. Neuroendocrine, biogenic amine and behavioral responsiveness to a repeated foot-shock-induced analgesia (FSIA) stressor in Sprague-Dawley (CD) and Fischer-344 (CDF) rats. *Brain Res.* 1986; 382:71–80. [PubMed: 2945619]
- Sghendo L, Mifsud J. Understanding the molecular pharmacology of the serotonergic system: using fluoxetine as a model. *J Pharm Pharmacol.* 2011; 64:317–325. [PubMed: 22309263]
- Sinclair-Worley L, Uphouse L. Effect of estrogen on the lordosis-inhibiting action of ketanserin and SB 206553. *Behav Brain Res.* 2004; 152:129–135. [PubMed: 15135976]

- Tao R, Fray A, Aspley S, Brammer R, Heal D, Auerbach S. Effects on serotonin in rat hypothalamus of D-fenfluramine, aminorex, phentermine and fluoxetine. *Eur J Pharmacol.* 2002; 445:69–81. [PubMed: 12065196]
- Tavoulari S, Forrest LR, Rudnick G. Fluoxetine (Prozac) binding to serotonin transporter is modulated by chloride and conformational changes. *J Neurosci.* 2009; 29:9635–9643. [PubMed: 19641126]
- Uphouse L. Female gonadal hormones, serotonin, and sexual receptivity. *Brain Res Brain Res Rev.* 2000; 33:242–257. [PubMed: 11011068]
- Uphouse L, Andrade M, Caldarola-Pastuszka M, Jackson A. 5-HT_{1A} receptor antagonists and lordosis behavior. *Neuropharmacology.* 1996; 35:489–495. [PubMed: 8793912]
- Uphouse L, Andrade M, Caldarola-Pastuszka M, Maswood S. Hypothalamic infusion of the 5-HT_{2/1C} agonist, DOI, prevents the inhibitory actions of the 5-HT_{1A} agonist, 8-OH-DPAT, on lordosis behavior. *Pharmacol Biochem Behav.* 1994; 47:467–470. [PubMed: 8208763]
- Uphouse L, Caldarola-Pastuszka M. Female sexual behavior following intracerebral infusion of the 5-HT_{1A} agonist, 8-OH-DPAT, into the medial preoptic area. *Brain Res.* 1993; 601:203–208. [PubMed: 8431766]
- Uphouse, L.; Guptarak, J. Serotonin and Sexual Behavior. In: Müller, C.; Jacobs, B., editors. *Handbook of the Neurobiology of Serotonin.* New York: Academic Press; 2010. p. 346-366.
- Uphouse L, Hensler JG, Sarkar J, Grossie B. Fluoxetine disrupts food intake and estrous cyclicity in Fischer female rats. *Brain Res.* 2006; 1072:79–90. [PubMed: 16423328]
- Uphouse L, Maswood S, Jackson A, Brown K, Prullage J, Myers T, Shaheen F. Strain differences in the response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT. *Pharmacol Biochem Behav.* 2002; 72:533–542. [PubMed: 12175449]
- White S, Uphouse L. Estrogen and progesterone dose-dependently reduce disruptive effects of restraint on lordosis behavior. *Horm Behav.* 2004; 45:201–208. [PubMed: 15047015]
- Wolf A, Caldarola-Pastuszka M, DeLashaw M, Uphouse L. 5-HT_{2C} receptor involvement in female rat lordosis behavior. *Brain Res.* 1999; 825:146–151. [PubMed: 10216181]
- Wolf A, Caldarola-Pastuszka M, Uphouse L. Facilitation of female rat lordosis behavior by hypothalamic infusion of 5-HT(2A/2C) receptor agonists. *Brain Res.* 1998; 779:84–95. [PubMed: 9473601]
- Zar, J. *Biostatistical Analysis.* Upper Saddle River, New Jersey: Pearson Prentice Hall; 2010.

Highlights

1. Fischer and Sprague-Dawley females were compared for their response to ketanserin.
2. Sprague-Dawley rats were slightly more sensitive to the 5-HT_{2A/2C} receptor antagonist.
3. The IC₅₀ was similar for the two strains.
4. Fluoxetine and ketanserin were not additive in effect.

Figure 1. L/M ratio after varying doses of ketanserin treatment

Hormonally primed, ovariectomized rats were pretested for sexual behavior 4 to 6 hr after progesterone. Rats were then injected with deionized (DI) water or with 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin. Fifteen minutes later, rats were again tested for two consecutive 15 min intervals. Data are the mean $\pm$ SE L/M ratios for the two 15 min interval after injection with deionized water or ketanserin. Pretest L/M ratios (0.99 ± 0.003 and 0.98 ± 0.007 , respectively, for Fischer and Sprague-Dawley females) did not differ between strains. The Ns for Fischer rats for (DI) water or 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin, respectively, were 5, 8, 9, 6, 10, 5, 13 and 7. Ns for Sprague-Dawley rats, respectively, were 5, 8, 10, 6, 9, 5, 14 and 6. *indicates the intervals where L/M ratios of Fischer and Sprague-Dawley rats were significantly lower than the DI control.

Figure 2. Lordosis quality after ketanserin treatment

Data are the mean $\pm$ SE lordosis quality score for the pretest and two 15 intervals after treatment with ketanserin or deionized water (DI) and are for the same rats as in Figure 1. The Ns for Fischer rats for DI water or 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin, respectively, were 5, 8, 9, 6, 10, 5, 13 and 7. Ns for Sprague-Dawley rats, respectively, were 5, 8, 10, 6, 9, 5, 14 and 6. *indicates the intervals where lordosis quality of Fischer and Sprague-Dawley rats were significantly lower than the DI control.

Figure 3. Effects of ketanserin on proceptivity

Data are the percentage of rats showing proceptivity after the various doses of ketanserin treatment and for the same rats as in Figure 1. The inset shows the percentage of rats when collapsed across doses of ketanserin. *indicates significant decline in proceptivity relative to the DI control, within strain.

Figure 4. Ketanserin and fluoxetine are not additive for lordosis inhibition

Hormonally primed, ovariectomized rats were pretested for sexual behavior 4 to 6 hr after progesterone. Rats were then injected with 10 mg/kg fluoxetine or deionized water (DI). Fifteen minutes later DI injected rats were injected with 1 mg/kg ketanserin; fluoxetine-treated rats were injected with 1 mg/kg ketanserin or DI. This led to three groups, fluoxetine only, ketanserin only, fluoxetine and ketanserin. Fifteen minutes later, rats were again tested for 15 consecutive min. Data are the mean $\pm$ SE L/M ratios for the pretest and 15 consecutive min after injection. The Ns for Fischer rats given fluoxetine, ketanserin, or both drugs were 8, 7 and 11. Ns for Sprague-Dawley rats given fluoxetine, ketanserin, or both drugs were 7, 7 and 11. *indicates significant difference from the pretest within treatment, # indicates a difference from fluoxetine within strain.

Figure 5. Effects of fluoxetine, ketanserin, and their combination on proceptivity
Data are the percentage of rats showing proceptivity after the 3 treatments and are for the same rats as in Figure 4. Data are for the Pretest and the 15 min interval after treatment.
*indicates a significant difference from the pretest and fluoxetine, within strain.

Table 1
Treatment Effects on Lordosis Quality Scores

Strain	Treatment			
	Pretest	Fluoxetine 10 mg/kg	Ketanserin 1 mg/kg	Fluoxetine + Ketanserin
Fischer	2.81 ± 0.038	2.89 ± 0.044	2.81 ± 0.072	2.75 ± 0.071
Sprague-Dawley	2.83 ± 0.048	2.71 ± 0.122	2.73 ± 0.129	2.31 ± 0.192 ^{a,b}

^a Indicates significantly different from Fischer within treatment

^b Indicates significantly different from all other groups within strain