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Restraint accentuates the effects of 5-HT_2 receptor antagonists and a 5-HT_{1A} receptor agonist on lordosis behavior

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Abstract

The effect of restraint on lordosis behavior was examined in proestrous and ovariectomized, hormone-primed rats. Restraint durations from 5 to 60 min had no effect on lordosis behavior of proestrous rats. There was also no effect of 5 min restraint on lordosis behavior of ovariectomized rats hormonally primed with 10 μg estradiol benzoate and 500 μg progesterone. However, after intraperitoneal treatment with 1.0 mg/kg ketanserin tartrate (ketanserin), 5 min of restraint significantly reduced lordosis behavior of both groups of rats. The 5-min restraint combined with 0.50 or 0.75 mg/kg ketanserin reduced lordosis to mount (L/M) ratios of ovariectomized rats, while L/M ratios of proestrous rats were inhibited only by the 1.0 mg/kg dose. Increasing the restraint duration (10 or 15 min) reduced the dose of ketanserin necessary to reduce the L/M ratios of proestrous rats. Treatment with the selective serotonin $(5\text{-HT})_{2C}$ receptor antagonist, SB206553 (2.5 or 5.0 mg/kg), in combination with 5 min of restraint, also reduced L/M ratios of hormonally primed, ovariectomized rats. The neural sites responsible for ketanserin's additivity with restraint are unknown, but infusion of the drug into the ventromedial nucleus of the hypothalamus (VMN) did not mimic the systemic treatment. However, 5 min of restraint did enhance the effects of VMN infusion with the 5-HT_{1A} receptor agonist, 8-OH-DPAT. In contrast, 8-OH-DPAT's systemic potency was not enhanced by restraint. These findings support the hypothesis that a mild stressor increases the lordosis-inhibiting effects of 5-HT_{1A} receptor agonists and that 5-HT_2 receptors may protect against such disruption of lordosis behavior.

Keywords: Stress; Female rats; Serotonin; Ovariectomized rats; Sexual receptivity

1. Introduction

In many species, female sexual behavior exerts critical control over reproductive fitness. In rats and mice, ovulatory cycles occur every 4–5 days and sexual receptivity by the female is tightly coupled to ovulation (Beach, 1976; Sodersten, 1981; Young, 1961). Coordination between reproductive behavior and physiological readiness to procreate is the result of female gonadal hormones acting at both CNS and peripheral sites (Clemens and Weaver, 1985) and includes modulation of and by multiple neurotransmitter systems (Auger et al., 2001; Luine et al., 1999; Etgen et al., 1992; McCarthy et al., 1991; Meyerson et al., 1985; Schiml and Rissman, 2000).

Female gonadal hormones exert a complex alteration of the serotonergic system that includes modulation of neurotransmitter receptors, serotonin transporter, and neurotransmitter synthesis, degradation, and release (Bethea et al., 2002; Mendelson, 1992; Uphouse, 2000). In general, an increase in 5-HT activity is associated with a reduction in female rat sexual receptivity as measured by the lordosis reflex (Allen et al., 1993; Frankfurt et al., 1994; Luine et al., 1984; Mendelson, 1992; Meyerson, 1964). The lordosis reflex is a supraspinal reflex, leading to arching of the back and assumption of a mating posture, and is essential for successful mating to occur. That estrogen and progesterone control the timing of lordosis behavior is quite clear, but only estrogen is required for the female to execute the lordosis posture (Clemens and Weaver, 1985; Pfaff, 1970; Sodersten, 1981). Progesterone's role is facilitatory to the reflex in that progesterone increases lordosis to mount (L/M) ratios when combined with relatively low doses of estrogen and it is this estrogen–progesterone synergy that is likely to control lordosis behavior in naturally cycling animals (Sodersten,

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1981). However, after estrogen priming, progesterone also enhances aspects of the female's reproductive behavior (e.g. proceptivity) that may be more closely equated with motivation or willingness to mate (Sodersten, 1981).

An intriguing aspect of serotonin's (5-HT) modulation of female sexual behavior is the robustness with which the behavior can be reduced by 5-HT [(presumably acting via 5-HT_{1A} receptors in the ventromedial nucleus of the hypothalamus (VMN)] (Aiello-Zaldivar et al., 1992; Uphouse et al., 1992a,b, 1993), while 5-HT_2 receptor-preferring compounds increase the behavior (Caldwell and Albers, 2002; Gonzalez et al., 1997; Mendelson and Gorzalka, 1985; Uphouse et al., 1996; Wolf et al., 1998). Yet, the importance of this putative facilitatory component to 5-HT's modulation of lordosis behavior is unclear since destruction of 5-HT neurons with 5,7-DHT does not prevent hormonal priming of the behavior (Frankfurt et al., 1985). In fact, destruction of 5-HT neurons reduces the dose of estrogen that is required for effective priming to take place (Frankfurt et al., 1985; Luine et al., 1984). This leads to the suggestion that 5-HT's inhibition of the reflex may be the primary role of the neurotransmitter.

Uphouse (2000) suggested that the apparent antagonism between the inhibitory 5-HT_{1A} receptors and the putative facilitatory 5-HT_2 receptors could allow the female to coordinate her behavior with environmental conditions. For example, should the female encounter a potentially threatening situation, stress-induced activation of 5-HT neurons would increase 5-HT release and activation of 5-HT_{1A} receptors and thereby reduce the readiness of the female to mate. However, since an unfamiliar, sexually active male might also provoke activation of the 5-HT system and a reduction of mating behavior, some mechanism must be available to prevent activation of the 5-HT_{1A} receptor-mediated stop signal or to reduce its duration to novel, mild, or transient stressful events. Such an attenuation of the stop signal could reside with 5-HT_2 receptors. Support for this idea comes from prior findings that 5-HT_2 receptor agonists can prevent the effects of 5-HT_{1A} receptor agonists on lordosis behavior (Maswood et al., 1996; Uphouse et al., 1994).

In the following experiments, we have tested the hypothesis that a mild, transient stress can reduce lordosis behavior in sexually receptive females under conditions of reduced 5-HT_2 receptor activation. We also evaluated the hypothesis that experience with a mild stressor would accentuate the effects of a 5-HT_{1A} receptor agonist.

2. Materials and methods

2.1. Materials

Disposable Decapicone® restrainers were purchased from Braintree Scientific (Braintree, MA). The 5-$HT_{2A/2C}$ receptor antagonist, 3-[2-[4-(4-fluorbenzoyl)-1-piperdinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione (ketanserin tartrate, hereafter referred to as ketanserin), the selective 5-HT_{2C} receptor antagonist, *N*-3-pyridinyl-3,5-dihydro-5-methyl-benzo(1,2-b:4,5-b′)dipyrrole-(2*H*)carboxamide (SB206553), the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), estradiol benzoate, progesterone, and sesame seed oil were purchased from Sigma Chemical (St. Louis, MO). Methoxyflurane (Metofane) and isoflurane (AErrane) were obtained from Pitman Moore (Mundelein, IL). Suture materials were purchased from Butler (Arlington, TX). All other materials were purchased from Fisher Scientific (Houston, TX).

2.2. General methods

2.2.1. Animals and housing

Female (CDF-344) rats were purchased as adults or were bred in the TWU animal facility from stock obtained from Sasco Laboratories (Wilmington, MA). Rats were housed two or three per cage in polycarbonate shoebox cages in a colony room maintained at 25 °C and 55% humidity, with lights on from 12 midnight to 12 noon. Food and water were available ad libitum. All procedures were conducted according to PHS policy and were approved by the IACUC at Texas Woman's University.

2.2.2. Surgical procedures and hormone treatments

Intact, proestrous females and ovariectomized, hormone-primed rats were used in the experiments. Vaginal smears of intact females were monitored as previously described (Uphouse et al., 1992b) for at least two complete estrous cycles. Females were selected for use in the experiments on the basis of their smear history and vaginal smear on the day of testing. Smears containing nucleated and/or cornified epithelial cells but no leukocytes were considered to be proestrous smears.

When ovariectomy was to be performed, rats were anesthetized with Metofane or AErrane and were ovariectomized as previously described (Uphouse et al., 1992b). Two weeks later, females were injected with 10 μg of estradiol benzoate followed 48 h later with 500 μg of progesterone or the sesame seed oil vehicle. Hormone injections were given subcutaneously in a volume of 0.1 ml per rat.

For implantation of intracranial cannulae, rats weighing between 140 and 170 g were anesthetized with AErrane and implanted bilaterally with 22-gauge stainless steel guide cannulae advanced stereotactically toward the VMN (atlas coordinates AP 4.38; DV ± 7.8; ML ± 0.4 from Konig and Klippel, 1963) as previously described (Uphouse et al., 1996). Ovariectomy was performed 2 weeks after implant surgery.

2.2.3. Testing for sexual receptivity

On the morning of testing (prior to lights out), rats were moved to the testing room where the males were housed. Testing for sexual behavior, as previously described

(Uphouse et al., 1992b), was initiated within 1–3 h after colony lights off and 4–6 h after the progesterone injection. Visibility was aided by red lighting. In the pretest for sexual receptivity, females were placed in the home cages of sexually experienced Sprague–Dawley male rats and behavior was monitored until the male had accomplished 10 mounts; rats with a pretest L/M ratio of 0.7 or higher were included in the remaining experiments. Lordosis quality, as previously described (Hardy and DeBold, 1971; Uphouse et al., 1992a,b), was also recorded.

After the pretest, rats were either placed in Decapicones® for the appropriate time interval or were returned to their home cage for a comparable interval. Rats were treated with pharmacological agents prior to the restraint or home cage experience. Following the restraint or home cage interval, the female was returned to the home cage of a sexually active male and behavior was monitored thereafter.

2.2.4. Restraint procedures

For restraint, the female was placed head first into the Decapicone® so that her nose was flush with the small opening at the tip of the cone. The base of the cone was then gathered around the female's tail and secured tightly with tape. When rats with intracranial cannulae were used, a longitudinal slit was made along the cone to allow room for the tops of the guide cannulae. The slit was then secured with lab tape after the female's nose was flush with the air hole. With this procedure, the female was tightly wrapped within the cone and was unable to turn. Generally, the process of wrapping the female required between 30 and 60 s. The wrapped female was set aside for the appropriate time interval.

2.2.5. Drug treatments

Ketanserin and SB206553 were dissolved in deionized water and 8-OH-DPAT was dissolved in saline. All systemic injections were in a volume of 1.0 ml/kg body weight and were given either intraperitoneally (ketanserin and SB206553) or subcutaneously (8-OH-DPAT). For intracranial treatments, drugs were infused at a rate of 0.24–0.26 μl/min to a final infusion volume of 0.5 μl per cannula. Intracranial drug doses are indicated as the amount of drug infused per cannula or one-half the dose per animal. Controls for systemic or intracranial treatments were with the appropriate vehicle.

2.2.6. Statistical procedures

For statistical purposes, L/M ratios or lordosis quality scores were grouped into the pretest interval and 5-min intervals after treatment. Data were evaluated by repeated-measures ANOVA with the number of main effects appropriate for the specific experiment. Differences between treatment groups, within time intervals, were compared with Tukey's test. Comparisons within groups, across time after treatment, were made with Dunnett's test to the pretest interval. The statistical reference was Zar (1999), and an alpha level of .05 was required for rejection of the null hypothesis. Since lordosis quality was unaffected by any of the experimental treatments, only experimental effects on L/M ratios are described.

2.3. Specific methods

2.3.1. Effects of restraint in proestrous rats

Thirty-seven proestrous female rats were used to evaluate the effects of mild restraint on lordosis behavior. After the pretest for sexual receptivity, proestrous females were restrained for 5, 15, 30, or 60 min, or were not restrained but were left in their home cage for the same time intervals. Immediately after restraint or the home cage experience, females were put back into the male's cage and behavior was monitored for three consecutive 5-min intervals. Females were then returned to their home cage and tested again for 10 mounts 15 min later (30 min after the restraint/home cage experience). Home cage controls (no restraint) were combined for inclusion in the analysis.

2.3.2. Effects of systemic treatment with 5-$HT_{2A/2C}$ receptor antagonists

Thirty-six proestrous female rats and 77 ovariectomized rats, hormonally primed with 10 μg estradiol benzoate and 500 μg progesterone, were used to evaluate the ability of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, to accentuate the effects of restraint. Females were pretested for sexual receptivity as described above. The female was then injected intraperitoneally with 0.50, 0.75, or 1.0 mg/kg ketanserin or an equivalent volume of deionized water. The female was then restrained or returned to the home cage for 5 min. Sexual behavior was monitored for three consecutive 5-min intervals and again 30 min after the experience as described earlier.

2.3.3. Effects of a 5-$HT_{2A/2C}$ receptor antagonist on lordosis behavior after longer durations of restraint

Thirty-three proestrous female rats were used to evaluate the effects of a 5-$HT_{2A/2C}$ receptor antagonist on lordosis behavior after longer duration of restraint. After the pretest for sexual receptivity, females were injected intraperitoneally with 0.75 mg/kg ketanserin and were restrained for 5, 10, or 15 min. An additional group of rats were returned to their home cage following the ketanserin injection. Sexual behavior was monitored as described above.

2.3.4. Effects of a 5-HT_{2C} receptor antagonist and 5 min of restraint

Thirty-six ovariectomized rats were hormonally primed with 10 μg estradiol benzoate followed 48 h later with 500 μg progesterone. After the 10-mount pretest (4–6 h after progesterone), rats were injected intraperitoneally with 2.5 or 5.0 mg/kg SB206553 or an equivalent volume of vehicle. Fifteen minutes later, rats were restrained for 5 min or were returned to their home cage

for 5 min. Sexual behavior was monitored as previously described.

2.3.5. Effects of intracranial infusion with ketanserin and restraint

Ketanserin was dissolved in deionized water and was infused into the VMN at a concentration of 250, 1000, or 1500 ng ketanserin per bilateral cannula (500, 2000, or 3000 ng per animal). Thirty-three ovariectomized rats with bilateral VMN cannulae were used in the initial experiment with 250 ng ketanserin per cannula. Twenty-five rats were used in a follow-up experiment with 1000 ng ketanserin per cannula and fourteen rats were infused with 1500 ng ketanserin per cannula. Infusions occurred immediately after the pretest. Rats were then placed in Decapicones® or were returned to the home cage for 5 min. Thereafter, behavior was monitored for 30 consecutive minutes.

2.3.6. Effects of 8-OH-DPAT and restraint

Thirty-seven rats with bilateral VMN cannulae were used for the 8-OH-DPAT study. 8-OH-DPAT was infused into the VMN at a concentration of 25 ng per bilateral cannula (50 ng per animal). Sixty-four rats were included in the study of the systemic effects of 8-OH-DPAT. Systemic injections of 8-OH-DPAT were given subcutaneously in a volume of 0.1 ml/100 g rat. 8-OH-DPAT doses of 0.0125 mg/kg, 0.025 mg/kg and 0.05 mg/kg were used.

3. Results

3.1. Effects of restraint in proestrous rats

L/M ratios (Fig. 1) of proestrous rats were unaffected by any of the restraint durations [ANOVA for treatment, respectively, $F(4,32)=.43$, $P>.05$]. However, when rats were injected with ketanserin immediately prior to a 5-min restraint, there were significant decrements in lordosis behavior (Fig. 2). As is evident in Fig. 2, in the absence of restraint, this dose of ketanserin had no effect on lordosis behavior; these data are in agreement with Uphouse et al. (1996) who reported that doses of 2 mg/kg ketanserin or more reduced L/M ratios of sexually receptive rats.

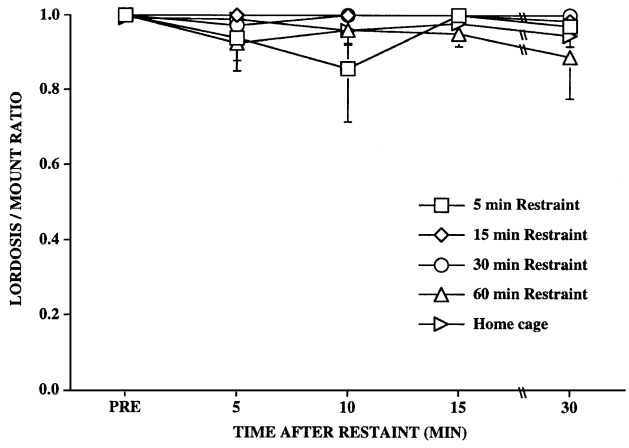

Fig. 1. Lordosis behavior of intact rats after restraint. Proestrous rats were pretested for sexual behavior and were then restrained for 5 ($n=7$), 15 ($n=6$), 30 ($n=4$), or 60 ($n=5$) min. Sixteen rats were not restrained but were returned to the home cage for comparable durations. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and each of the test intervals after the restraint or home cage control.

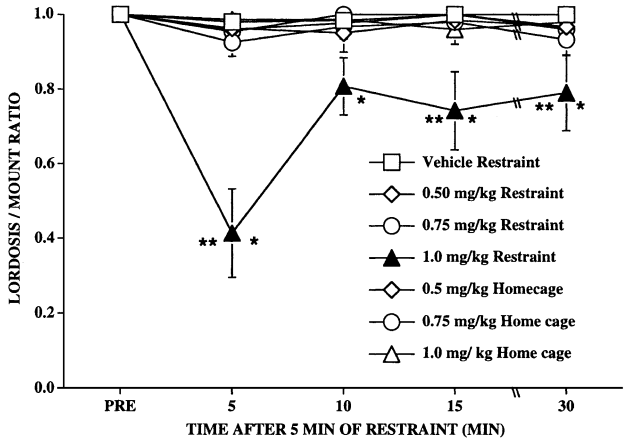

Fig. 2. Effects of ketanserin on lordosis behavior of proestrous females after restraint. Proestrous rats were pretested for sexual behavior. Rats were then injected intraperitoneally with ketanserin or vehicle and either restrained for 5 min or returned to the home cage. Immediately after the appropriate experience, sexual behavior of the females was monitored as described in Materials and Methods. Shown in the figure are the mean ± S.E. L/M ratios for the pretest (PRE), three 5-min intervals after restraint, and a single test interval 30 min after restraint. *N*'s for rats that were given 0.50, 0.75, 1.0 mg/kg ketanserin, or vehicle and restrained for 5 min were 5, 6, 6, and 5, respectively. *N*'s for rats that were given 0.50, 0.75, or 1.0 mg/kg ketanserin and returned to the home cage, respectively, were 3, 5, and 5. Single asterisks indicate significant differences, within injection groups, from the appropriate pretest interval. Double asterisks indicate significant differences, within time intervals, from restrained rats that were injected with 1.0 mg/kg ketanserin.

In order to compare the effects of ketanserin in the home cage vs. the restraint experience, the vehicle group was excluded and data were compared with a two-way repeated-measures ANOVA (Dose of Drug × Type of Experience). There were significant main effects of restraint vs. no restraint (home cage) [$F(2,25)=12.60$, $P\leq.05$] and dose of ketanserin [$F(1,25)=17.45$, $P\leq.05$] on lordosis behavior. However, these main effects resulted exclusively from the effects of 1.0 mg/kg ketanserin plus restraint. For these rats, there was a significant decrease in the L/M ratio by 5 min after restraint [Dunnett's $q(100,6)\geq 2.55$, $P\leq.05$]. None of the other treatments significantly decreased lordosis behavior after restraint [Dunnett's $q(100,6)\leq 2.55$, $P\geq.05$] and all other treatments were significantly different from 1.0 mg/kg ketanserin plus restraint [Tukey's $q(100,6)\geq 4.10$, $P\leq.05$].

Although the L/M ratio of rats injected with 1.0 mg/kg ketanserin and restrained for 5 min began to increase by 10 min after restraint, L/M ratios remained significantly

lower than the pretest at all time intervals [Dunnett's $q(100,6) \geq 2.55$, $P \leq .05$]. As a consequence, there was a significant effect of time after the experience [$F(4,100) = 5.12$, $P \leq .05$], as well as a significant interaction between time and type of experience [$F(4,100) = 2.90$, $P \leq .05$] and between time and dose of ketanserin [$F(8,100) = 2.39$, $P \leq .05$].

A separate one-way, repeated-measures ANOVA was performed with the vehicle group included and the home cage group excluded in order to confirm the dose-dependent effects of ketanserin in the restraint condition. Consistent with the prior analysis, there was a significant effect of the treatment on L/M ratios [$F(3,19) = 19.18$, $P \leq .05$]. Vehicle plus 5 min of restraint had no effect on the L/M ratios (all $q \leq 2.41$, $P \geq .05$) but there was a decline in the L/M ratio when rats were restrained after injection with 1.0 mg/kg ketanserin. This decrease was evident at each time point after restraint [Dunnett's $q(76,4) \geq 2.41$, $P \leq .05$]. Consequently, there were significant effects of time after experience [$F(4,76) = 6.00$, $P \leq .0003$] and the interaction between time and treatment was also significant [$F(12,76) = 3.80$, $P \leq .05$].

3.2. Effects of 5-HT$_2$ receptor antagonists in hormone-primed, ovariectomized rats

Ovariectomized rats, hormonally primed with 10 μg estradiol benzoate and 500 μg progesterone were not affected by 5 min of restraint, but, similar to proestrous females, ketanserin plus restraint inhibited lordosis behavior (Fig. 3). However, in contrast to the effects of ketanserin in proestrous females, all doses of ketanserin led to some decline in lordosis behavior after the 5-min restraint experience.

As described above, data were compared with a two-way, repeated-measures ANOVA (Dose of Drug × Type of Experience) with the vehicle group excluded in order to evaluate the effects of ketanserin in the restraint vs. the home cage experience. Data were also compared with a one-way, repeated-measures ANOVA (with the home cage group excluded and the water group included) to evaluate the dose-dependent effects of ketanserin in the restraint condition. Consistent with the findings in proestrous females, there were significant effects of restraint vs. no restraint (home cage) [$F(1,57) = 12.12$, $P \leq .05$] and dose of [$F(2,57) = 3.18$, $P \leq .05$] on lordosis behavior. There was also a significant effect of time after the experience [$F(4,228) = 16.89$, $P \leq .0001$], as well as a significant interaction between time and type of experience [$F(4,228) = 6.08$, $P \leq .0001$] and between time and dose of ketanserin [$F(8, 228) = 2.94$, $P \leq .005$]. When the vehicle group plus restraint was included in the ANOVA of restrained rats, there was a significant effect of drug treatment [$F(3,47) = 4.45$, $P \leq .008$], time after treatment [$F(4,188) = 21.28$, $P \leq .0001$], and time after treatment × drug treatment interaction [$F(12,188) = 3.39$, $P \leq .0002$].

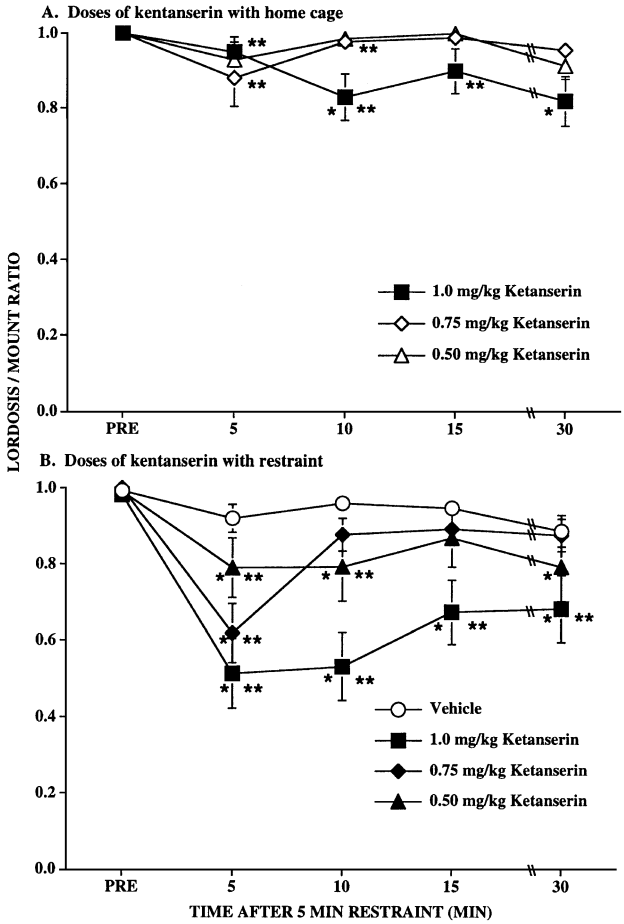

Fig. 3. Ketanserin accentuates effects of restraint in hormone-primed ovariectomized rats. Ovariectomized female rats were hormonally primed and pretested (PRE) for sexual behavior as described in Materials and Methods. Rats were injected intraperitoneally with ketanserin or vehicle and were either restrained for 5 min or returned to their home cage for 5 min. The mean ± S.E. L/M ratios for rats that received 0.5, 0.75, or 1.0 mg/kg ketanserin and were returned to their home cage are shown in A. *N*'s, respectively, were 8, 8, and 10. The mean ± S.E. L/M ratios for rats that received 0.50, 0.75, or 1.0 mg/kg ketanserin or vehicle and were restrained for 5 min are shown in B. *N*'s, respectively, were 13, 11, 13, and 14. Single asterisks indicate where the L/M ratio was significantly different from the pretest interval. Double asterisks in A indicate intervals where home cage and restrained rats differed significantly within treatment condition. Double asterisks in B indicate intervals where restrained rats given ketanserin differed significantly from the restrained vehicle control.

3.3. Effects of longer durations of restraint in proestrous rats

In the next experiment, we considered if longer restraint durations would enhance the proestrous female's vulnerability to ketanserin plus restraint. As shown in Fig. 4, with longer restraint intervals, proestrous females showed a decline in lordosis behavior following 0.75 mg/kg ketanserin.

There was a significant effect of treatment [$F(3,29) = 4.90$, $P \leq .0001$] and a significant treatment by time interaction [$F(12,116) = 4.62$, $P \leq .0001$]. Rats that were restrained for 10 or 15 min and given 0.75 mg/kg ketanserin showed a

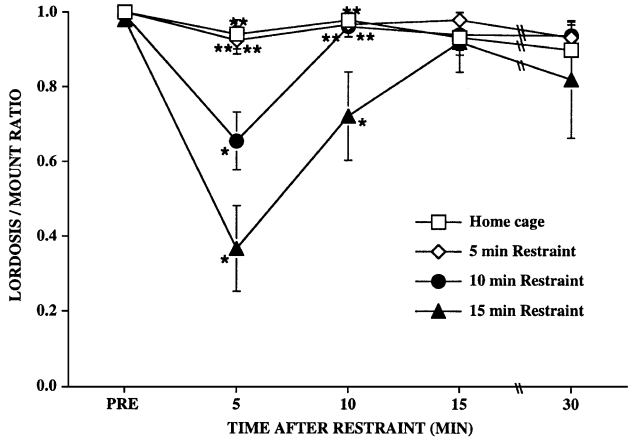

Fig. 4. Effects of ketanserin on lordosis behavior after longer durations of restraint. Proestrous rats were pretested for sexual behavior. They were then injected intraperitoneally with 0.75 mg/kg ketanserin and were restrained for 5 ($n=6$), 10 ($n=13$), or 15 ($n=5$) min. An additional of nine rats were not restrained but were returned to their home cage following the ketanserin injection. After the appropriate experience, rats were returned to the male's cage for sexual behavior testing. Shown in the figure are the mean ± S.E. L/M ratios for the pretest (PRE), three 5-min intervals after restraint, and a single test interval 30 min after restraint. Single asterisks indicate significant differences, within restraint groups, from the appropriate pretest interval. Double asterisks indicate significant differences, within time intervals, from rats that were restrained for 10 or 15 min.

significant decrease in the L/M ratio by 5 min after restraint [Dunnett's all $q(116,4) \geq 7.84$, $P \leq 0.05$]. The L/M ratio increased by 10 min after restraint, but the L/M ratio of rats restrained for 15 min remained significantly lower than the pretest at this time [Dunnett's $q(116,4)=2.38$, $P \leq .05$]. All other treatments were significantly different from rats restrained for 10 or 15 min and given 0.75 mg/kg ketanserin [Tukey's all $q(116,4) \geq 3.69$, $P \leq .05$].

3.4. Effects of the selective 5-HT$_2$ receptor antagonist, SB206553

In the next experiment, a more selective 5-HT$_2$ receptor antagonist, SB206553, with preference for the 5-HT$_{2C}$ receptor was examined (Fig. 5). Since effective systemic doses of this drug for decreasing lordosis behavior were not known, in a separate experiment, ovariectomized females were tested for their response to 2.5 or 5.0 mg/kg SB206553. Fourteen rats were injected immediately after the pretest and sexual behavior was monitored 15 min after the injection (data not shown). Since L/M ratios of rats injected with 2.5 mg/kg SB206553 never decreased below 0.8, this dose was selected as the subthreshold dose for the current study. As shown in Fig. 5, restraint enhanced the effect of SB206553. When the vehicle group was excluded in order to statistically compare the effects of SB206553 in the home cage vs. the restraint experience, there were significant effects of restraint vs. no restraint (home cage) [$F(1,24)=21.53$, $P \leq .05$] and dose of SB206553 [$F(1,24)=13.99$, $P \leq .05$] on lordosis behavior. There was also a significant effect of time after the experience [$F(4,100)=116.43$, $P \leq .05$], as well as a significant interaction between time and type of experience [$F(4,100)=8.56$, $P \leq .05$] and between time and dose of SB206553 [$F(4,100)=6.90$, $P \leq .05$].

When data were compared with the home cage group excluded, there was a significant effect of the dose of SB206553 on L/M ratios after restraint [$F(2,19)=19.00$, $P \leq .05$]. Vehicle plus 5 min restraint had no effect on the L/M ratios. However, 5 min after restraint, there was a significant decline in the L/M ratio after injection with 2.5 or 5.0 mg/kg SB206553 [Dunnett's $q(76,3)=2.27$, $P \leq .05$].

3.5. Effects of intracranial infusion with ketanserin

Ketanserin was infused into the VMN to test the hypothesis that antagonism of 5-HT$_2$ receptors in this brain area was responsible for the effects of the drug seen after systemic injection. In the initial experiment, a subthreshold dose of 250 ng ketanserin per cannula was chosen on the basis of prior studies that this dose of the drug has little effect on lordosis behavior of sexually receptive rats (Uphouse et al., 1996). When restraint was compared in vehicle vs. ketanserin-infused rats, there was no significant effect of the type of treatment on L/M ratios [$F(2,30)=1.13$, $P>.05$] (Fig. 6). Although time after the experience was a significant factor [$F(6,180)=8.63$, $P \leq .0001$], the interaction between time and type of experience was not significant

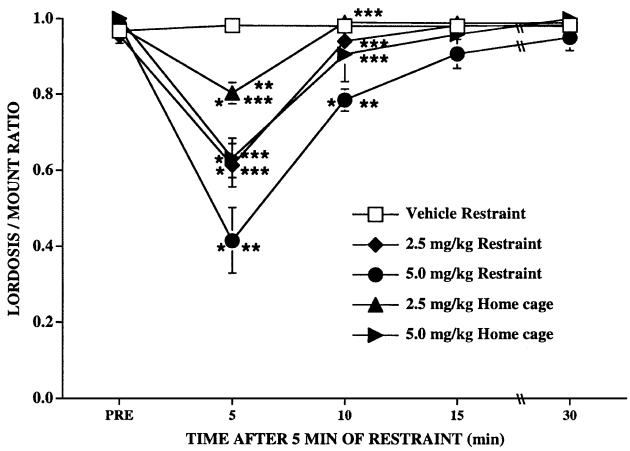

Fig. 5. Effects of SB206553 on lordosis behavior of ovariectomized females after restraint. Ovariectomized rats were hormonally primed, tested for sexually receptivity, and injected intraperitoneally with SB206553 or vehicle. Fifteen minutes later, females were either restrained or returned to the home cage for 5 min. Shown in the figure are the mean ± S.E. L/M ratios for the pretest (PRE), three 5-min intervals after restraint, and a single test interval 30 min after restraint. *N*'s for rats given 2.5 or 5.0 mg/kg SB206553, or vehicle, respectively, were 10, 6, and 6 for the restraint group and 9 and 5, respectively, for rats given SB206553 and returned to the home cage. Single asterisks indicate significant differences, within injection groups, from the appropriate pretest interval. Double asterisks indicate times points where, within time intervals, rats differed from restrained rats that were injected with 2.5 mg/kg SB206553. Triple asterisks indicate times points where, within time intervals, rats differed from restrained rats that were injected with 5.0 mg/kg SB206553.

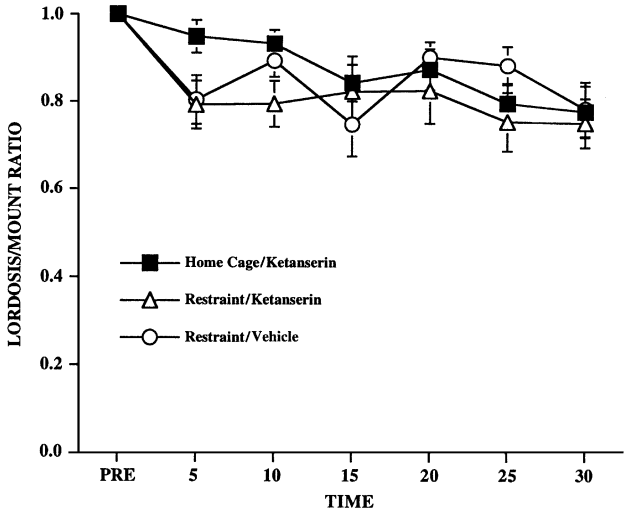

Fig. 6. Effects of ketanserin infusion into the VMN on the response to 5 min of restraint. Ovariectomized rats with bilateral VMN implants were injected with 10 μg estradiol benzoate followed 48 h later with 500 μg progesterone. Four to six hours later, rats were given one of the following three treatments: (a) 5 min restraint with water (vehicle) infusion ($n=11$), (b) 5 min restraint with 250 ng ketanserin infusion ($n=10$), or (c) 250 ng ketanserin infusion and 5 min return to the home cage ($n=12$). Data are the mean ± S.E. L/M ratios for the pretest (PRE) and for consecutive 5-min intervals after the restraint or home cage experience.

[$F(12,180)=1.27$, $P>.05$] and the overall time-dependent decline in L/M ratios was small.

Since 250 ng ketanserin per cannula produced only small effects in any of the treatment conditions, two additional experiments were performed with infusion of 1000 or 1500 ng ketanserin per cannula (Fig. 7). Although these higher doses of ketanserin produced a substantial decline in lordosis behavior [for 1000 and 1500 respectively, $F(6,138)=7.73$ and $F(6,72)=4.95$, $P\leq.0001$], there was no effect of the type of experience on this decline and there was no interaction between experience and time after the experience (all $P>.05$).

3.6. Effects of 8-OH-DPAT and restraint

On the basis of prior experiments (Uphouse et al., 2002; Truitt et al., 2003), a subthreshold dose of 25 ng 8-OH-DPAT per cannula was chosen for infusion into the VMN. Consistent with these prior studies, 8-OH-DPAT did not reduce L/M ratios of rats that were returned to the home cage. However, 8-OH-DPAT plus 5 min of restraint reduced L/M ratios throughout the test interval (Fig. 8). There were significant effects of 8-OH-DPAT vs. vehicle [$F(1,23)=7.88$, $P\leq.01$], time after infusion [$F(6,138)=5.03$, $P\leq.0001$], and the interaction between time after infusion and type of infusion [$F(6,138)=2.59$, $P\leq.03$]. 8-OH-DPAT plus restraint was the only treatment condition for which there was a significant decline in L/M ratios. For this condition, L/M ratios of every posttreatment test interval were significantly different from the pretest interval [Dunnett's all $q(138,7)\geq2.57$, all

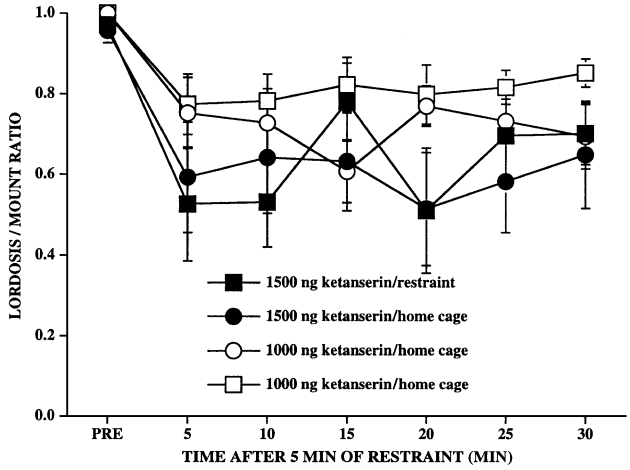

Fig. 7. Effects of higher doses of VMN ketanserin and restraint. Ovariectomized rats with bilateral VMN implants were treated as described in Fig. 6 but were infused with 1000 or 1500 ng ketanserin per bilateral cannulae. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and for consecutive 5-min intervals after the restraint or home cage experience. *N*'s for 1000 and 1500 ng ketanserin for the restraint and home cage condition, respectively, are 7, 7, 12, and 13.

$P\leq.05$]. Differences between restrained rats infused with 8-OH-DPAT and restrained rats infused with vehicle were most prominent during the first 15 min where the two groups were significantly different at each test interval [Tukey's all $q(138,4)\geq3.66$, $P\leq.05$]. Similarly, the effects of restraint in 8-OH-DPAT-treated rats were most evident during the first 15 min after restraint [8-OH-DPAT restrained vs. 8-OH-DPAT home cage differed significantly at 5 and 15 min after infusion, Tukey's all $q(138,4)\geq3.66$, $P\leq.05$].

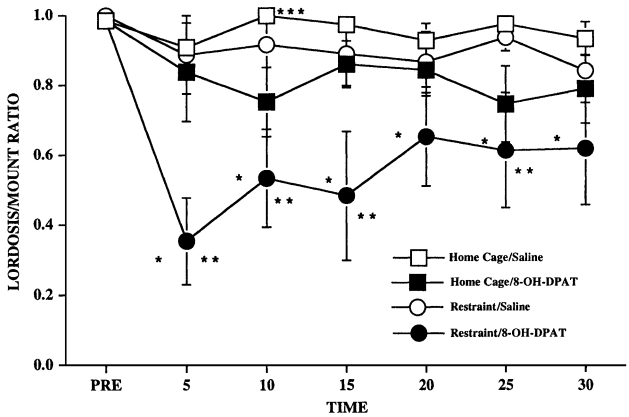

Fig. 8. Effects of 8-OH-DPAT infusion into the VMN on the response to 5 min of restraint. Ovariectomized rats with bilateral VMN implants were injected with 10 μg estradiol benzoate followed 48 h later with 500 μg progesterone. Four to six hours later, rats were infused with vehicle ($n=6$) or 8-OH-DPAT ($n=7$) and restrained for 5 min or infused with vehicle ($n=7$) or 8-OH-DPAT ($n=7$) and returned to the home cage for 5 min. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and for consecutive 5-min intervals after the restraint or home cage experience. A single asterisk indicates a difference between 8-OH-DPAT in the home cage vs. restraint condition; double and triple asterisks indicate differences between 8-OH-DPAT and vehicle in the restraint condition and home cage conditions, respectively.

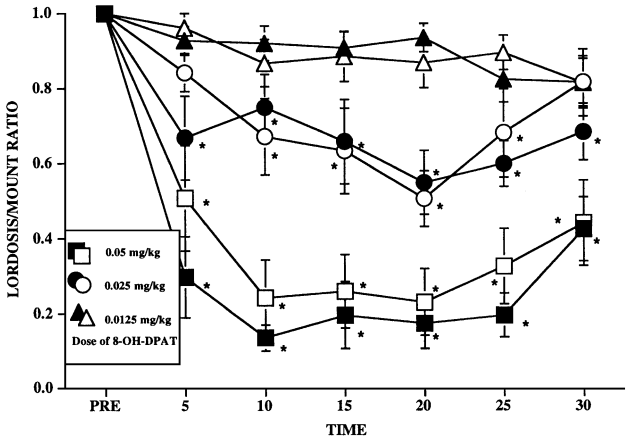

Fig. 9. Effects of systemic 8-OH-DPAT and 5 min restraint. Data are the mean ± S.E. L/M ratios for ovariectomized rats, hormonally primed with 10 μg estradiol benzoate and 500 μg progesterone. Rats were injected subcutaneously with varying doses of 8-OH-DPAT immediately prior to 5 min restraint or home cage experience as described in Materials and Methods. *N*'s for 0.0125, 0.025, and 0.05 mg/kg 8-OH-DPAT for the restraint condition, respectively, were 11, 10, and 11; *N*'s for 0.0125, 0.025, and 0.05 mg/kg 8-OH-DPAT for the home cage condition, respectively, were 10, 11, and 11. Filled symbols are for rats restrained for 5 min; open symbols indicate data for rats returned to the home cage. Single asterisks indicate intervals where the L/M ratio, within time interval, differed significantly from the pretest interval (PRE).

If, as hypothesized, restraint leads to increased 5-HT release in the VMN and this endogenous 5-HT sums with the exogenous 8-OH-DPAT infusion to activate sufficient 5-HT_{1A} receptors to inhibit lordosis behavior, then systemic treatment with a relatively low dose of 8-OH-DPAT should not show this interaction between restraint and 8-OH-DPAT. Activation of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN) should reduce release of endogenous 5-HT (Hjorth and Sharp, 1991). Consequently, effects of systemic 8-OH-DPAT, by activating somatodendritic 5-HT_{1A} autoreceptors in the DRN and 5-HT_{1A} receptors in the VMN, should be independent of restraint. This expectation was confirmed (Fig. 9). Consistent with prior observations (Uphouse et al., 2002), there was a dose-dependent reduction of lordosis behavior after 8-OH-DPAT [$F(2,58) = 53.08$, $P \leq .0001$], a significant effect of time after treatment [$F(6,348) = 37.17$, $P \leq .0001$], and a significant interaction between time and dose of 8-OH-DPAT [$F(12,348) = 9.15$, $P \leq .0001$]. However, the main effect of restraint vs. home cage was not significant [$F(1,58) = 0.882$, $P > .05$].

4. Discussion

These experiments were designed to test two specific hypotheses: (1) that 5-HT_2 receptors allow the female rat to continue mating following exposure to mild or transient stress, and (2) that mild stress accentuates the effect of a 5-HT_{1A} receptor agonist on lordosis behavior.

Although 5-HT_{1A} receptor agonists are known to inhibit female rat lordosis behavior (Ahlenius et al., 1986; Aiello-Zaldivar et al., 1992; Uphouse et al., 1992a,b), this is the first report in which the effect of a 5-HT_{1A} receptor agonist in combination with a mild stressful experience has been reported. The fact that 5 min of restraint experience was able to amplify the effects of the VMN infusion with the 5-HT_{1A} receptor agonist has implications for the role played by 5-HT_{1A} receptors in the modulation of female rat sexual behavior. Although hormonal changes during the female rat estrous cycle reduce the potency of 5-HT_{1A} receptor agonists in inhibiting lordosis behavior (Jackson and Uphouse, 1996, 1998), 8-OH-DPAT's ability to inhibit the behavior is not eliminated. These observations, coupled with findings that extracellular levels of 5-HT in the mediobasal hypothalamus decline during the period of sexual receptivity (Gereau et al., 1993; Farmer et al., 1996; Maswood et al., 1999), have led to conclusions that it is the extracellular 5-HT signal rather than the 5-HT_{1A} receptor response in the VMN that is altered as the female moves from the nonreceptive to the receptive state (Uphouse, 2000). If 5-HT_{1A} receptors in the VMN allow the female to reduce sexual receptivity, then the potential for activation of these receptors even during periods of heightened sexual arousal may enable the female to interrupt sexual receptivity in response to environmental conditions.

Stress impacts the functioning of the 5-HT system in a variety of brain regions. Of most relevance to the current findings, significant effects of restraint on 5-HT in lateral hypothalamus and VMN have been reported (Inoue et al., 1994; Shimizu et al., 1992); so it is not unreasonable to expect that the 5-min restraint experience would have transiently increased extracellular 5-HT, leading to occupation of 5-HT_{1A} receptors. When this increase in 5-HT was accompanied by infusion of a low dose of 8-OH-DPAT, sufficient 5-HT_{1A} receptors may have been occupied to produce inhibition of lordosis behavior. Without a putative stress-induced elevation of endogenous 5-HT, 25 ng of 8-OH-DPAT per cannula was insufficient.

Consequently, it is not surprising that the VMN infusion with 8-OH-DPAT was more effective than the systemic treatment at differentiating the restraint vs. the home cage condition. Following systemic administration of 8-OH-DPAT, somatodendritic 5-HT_{1A} receptors and 5-HT_{1A} receptors, postsynaptic to 5-HT neurons, are activated. Activation of postsynaptic 5-HT_{1A} receptors is responsible for the drug's inhibitory effect on lordosis behavior (Aiello-Zaldivar et al., 1992; Uphouse et al., 1992b), while activation of somatodendritic 5-HT_{1A} receptors in the dorsal raphe do not inhibit the behavior (Uphouse et al., 1992a). However, activation of somatodendritic 5-HT_{1A} receptors in the DRN does reduce extracellular 5-HT in terminal regions (Hjorth and Sharp, 1991). Therefore, systemic treatment with low doses of 8-OH-DPAT might reduce the restraint-induced increase in 5-HT and eliminate the cumulative effect of restraint and 8-OH-DPAT that was observed after VMN infusion with the drug.

We previously suggested that VMN 5-HT_{1A} receptors might function to enable the sexually receptive female to coordinate her behavior with environmentally relevant stimuli. Specifically, it was suggested that these receptors allow the female to reduce sexual behavior when environmental conditions are inappropriate or threatening. The current findings are consistent with that suggestion. The role played by 5-HT_2 receptors in the modulation of lordosis behavior have been more difficult to define but we have hypothesized that 5-HT_2 receptors in the VMN allow the female to reduce lordosis inhibition. The current findings lend only partial support to this hypothesis.

Systemic treatment with either the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, or the 5-HT_{2C} receptor antagonist, SB206553, accentuated the effects of 5 min restraint on lordosis behavior. Although SB206553 appeared to be more potent in its effects, this could be an artifact of having examined the drugs on different portions of their dose response curves. Since both drugs can reduce lordosis behavior after systemic or VMN infusion (Uphouse et al., 1996; Wolf et al., 1998; current manuscript), the lower doses of drugs used in this study were intended to be subthreshold for the inhibition of lordosis behavior. Nevertheless, the chosen doses were not equidistant from their respective threshold doses. Therefore, it also is not possible to conclude whether 5-HT_{2A} or 5-HT_{2C} receptors are responsible for the findings obtained in these studies. Although ketanserin has greater affinity for 5-HT_{2A} receptors, both 5-HT_{2A} and 5-HT_{2C} receptors are affected by the compound (Hoyer et al., 1987). Nevertheless, since the lordosis-inhibiting effects of the selective 5-HT_{2C} receptor antagonist, SB206553, were enhanced by restraint, it is likely that 5-HT_{2C} receptors contributed to the interaction between restraint and the 5-HT_2 receptor system.

Previously, we reported that lordosis behavior of ovariectomized rats made sexually receptive with estrogen (but not progesterone) was reduced by 5 min restraint (Truitt et al., 2003). Behavior of rats injected systemically with ketanserin or SB206553 prior to restraint closely resembled that of these estrogen-primed ovariectomized rats. These findings are interesting in view of prior suggestions that progesterone may enhance lordosis behavior, in part, by increasing activity at 5-HT_2 receptors (Wilson and Hunter, 1985).

Surprisingly, the effects of systemic treatment with ketanserin plus restraint were not mimicked following VMN infusion with ketanserin. We have previously reported that ketanserin infusion into the VMN reduces lordosis behavior of only about 50% of rats that are fully sexually receptive; in contrast, 100% of suboptimally hormone-primed ovariectomized rats show a decline in lordosis behavior (Uphouse et al., 1996; Uphouse, 2000). Thus, we had expected that restraint would accentuate the effects of VMN infusion with ketanserin, but this was not the case.

However, these studies do not rule out a physiologically significant involvement in the interaction between 5-HT_{1A} and 5-HT_2 receptors in the VMN. In prior experiments, it has been clearly established that the VMN is: (1) a primary site for 5-HT_{1A} receptor inhibition of lordosis behavior, (2) a site where 5-HT_2 receptor antagonists can reduce lordosis behavior, and (3) a site where 5-HT_2 receptor agonists can attenuate the effects of 5-HT_{1A} receptor agonists (Uphouse et al., 1992b, 1994, 1996). However, the *most* influential sites for 5-HT_2 receptor modulation of lordosis behavior have not been determined. Further studies should be directed toward this objective. Nevertheless, from the current studies, it can be concluded that restraint accentuates the effects of a 5-HT_{1A} receptor agonist in the VMN and that lordosis disruptive effects of 5-HT_2 receptor antagonists are enhanced by the stressful experience. However, sites in addition to the VMN may be involved.

Stress can disrupt reproductive functioning of the female rat (Owens and Nemeroff, 1991; Rivest and Rivier, 1995) due in part to a stress-induced disruption of gonadotrophin activity (Jeong et al., 1999; Rivest and Rivier, 1995). However, when chronic stress precedes hormonal priming, a resulting increase in sexual behavior has been attributed to enhanced 5-HT_{2A} receptor function (Gorzalka et al., 1998). There is little agreement about the effects of a relatively mild, acute stressor on gonadotrophin function and even less information about how an acute stress might influence the female's mating behavior. This is one of the first reports in which the immediate impact of stress on sexual receptivity has been examined in rats that are already fully sexually receptive. However, there have been several studies which have shown that an acute stress may advance the onset of sexual receptivity in naturally cycling female rats or in estrogen-primed, ovariectomized rats (Campbell et al., 1977; Gorzalka and Whalen, 1977; Nequin and Schwartz, 1971). Such advancement has been attributed, in part, to a stress-induced increase in progesterone secretion from the adrenals (Campbell et al., 1977; Gorzalka and Moe, 1994; Nequin and Schwartz, 1971).

It is surprising that so little attention has been paid to the acute effects of stress on reproductive behavior. This lack of attention contrasts with emphasis on the response of females to stress-induced hypothalamic–pituitary–adrenal (HPA) activation (Figueiredo et al., 2002; Haleem et al., 1989; Young et al., 1961), gonadal hormonal modulation of the HPA axis (Raap et al., 2000; Viau and Meaney, 1991), and to gender and/or estrous cycle differences in measures of anxiety (Karandrea et al., 2000; Kennett et al., 1986). In general, females have been reported to be more vulnerable than males to stress as measured by HPA activation (Fernandez-Guasti and Picazo, 1990; Kennett et al., 1986). Moreover, proestrous females are reported to have heightened HPA activation after stress relative to females at other stages of the estrous cycle (Critchlow et al., 1963; Viau and Meaney, 1991).

Such enhanced sensitivity to stress may be beneficial to the female's reproductive success. Even if the female were optimally sexually receptive, under life-threatening conditions, mating would be an inappropriate behavioral choice

for the female to make. Since the process of finding a mate and engaging in a complex mating sequence can expose the female to a potentially threatening environment, heightened responsivity to stressful situations might be expected to enhance the female's ability to survive to mate another day. However, should the threat be minimal, increased vulnerability to stress might unnecessarily reduce reproductive success. Coincident activation of 5-HT_2 receptors may prevent such reductions in sexual behavior.

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