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PROGESTERONE REDUCES THE EFFECT OF THE SEROTONIN 1B/1D RECEPTOR ANTAGONIST, GR 127935, ON LORDOSIS BEHAVIOR

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Abstract

Ovariectomized rats were hormonally primed with 10 µg estradiol benzoate or with estradiol benzoate plus 500 µg progesterone. Rats received a bilateral infusion with 200 ng of the 5-HT_{1B/1D} receptor antagonist, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1-1'-biphenyl-4-carboxamide hydrochloride (GR 127935), into the ventromedial nucleus of the hypothalamus (VMN), followed by a 5 min restraint or home cage experience. In estrogen-primed females that had experienced minimal handling between ovariectomy and use in the experiment, infusion with the water vehicle transiently inhibited lordosis behavior, and the 5-HT_{1B/1D} receptor antagonist amplified this inhibition. There were no effects in rats hormonally primed with estrogen and progesterone. Handling for two days before the experiment reduced the effects of the infusions in estrogen-primed rats. However, when a 5 min restraint experience followed infusion with GR 127935, there was a significant decline in lordosis behavior that persisted for 10 to 15 min after the experience. Regardless of the prior experience or type of infusion, the addition of progesterone to the hormonal priming completely prevented the lordosis inhibition. These findings are consistent with prior evidence that progesterone protects against the inhibitory effects of a 5 min restraint experience on lordosis behavior. Moreover, these are the first experiments to demonstrate an inhibitory effect of a selective 5-HT_{1B/1D} receptor antagonist in the VMN on lordosis behavior of estrogen primed, but not estrogen and progesterone primed, ovariectomized rats.

Keywords

stress; restraint; sexual behavior; reproduction; hypothalamus; ventromedial nucleus

Introduction

The lordosis reflex is a supraspinal reflex, made by sexually receptive female rodents in response to somatosensory stimuli by the male (Pfaff and Modianos, 1985). The reflex is orchestrated by estrogen and progesterone priming, but only estrogen is required (Pfaff and Modianos, 1985; Sodersten, 1981). Progesterone increases lordosis frequency when combined with relatively low doses of estrogen (Sodersten, 1981), but a variety of neurotransmitters, including serotonin (5-HT), influence whether or not lordosis behavior will occur (Bethea, et

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al., 2002; Etgen, et al., 2001; Fink, et al., 1996; Frye, 2007; Kow and Pfaff, 1985; McQueen, et al., 1997). Depending on the receptor subtype activated, 5-HT receptor agonists can either inhibit or facilitate lordosis behavior (Mendelson, 1992; Uphouse, 2000). Activation of 5-HT_{1A} receptors in the ventromedial nucleus of the hypothalamus (VMN) are primarily responsible for the lordosis-inhibiting effects of 5-HT (Uphouse, et al., 1996; Uphouse and Caldarola-Pastuszka, 1993). In contrast, activation of 5-HT_{2A/2C} receptors facilitates the behavior (Gonzalez, et al., 1997; Wolf, et al., 1998) and attenuates lordosis inhibition mediated by 5-HT_{1A} receptors in the VMN (Uphouse, et al., 1994). Hormonal priming that facilitates lordosis behavior reduces functioning of 5-HT_{1A} receptors (Mize and Alper, 2000; Mize, et al., 2003) and enhances that of 5-HT_{2A/2C} receptors (Ansonoff and Etgen, 1998; Cyr, et al., 1998; Gorosito and Cambiasso, 2008; Karp, et al., 2007; Marino, et al., 2006; Sumner and Fink, 1995; Sumner and Fink, 1997).

Nevertheless, in a naturally cycling rat, 5-HT_{1A} receptor agonists continue to be effective in reducing lordosis behavior of sexually receptive, proestrous female rats (Uphouse, 2000). Therefore, additional mechanisms must be involved to reduce 5-HT_{1A} receptor-mediated inhibition so that sexual behavior emerges. One such mechanism may reside in the modulation of extracellular 5-HT in the mediobasal hypothalamus (MBH). As the naturally cycling female moves toward the sexually receptive state, there is a decline in extracellular 5-HT in the mediobasal hypothalamus (MBH) that appears to result from the effects of progesterone (Farmer, et al., 1996; Gundlach, et al., 1998; Maswood, et al., 1999). Consequently, while estrogen may reduce 5-HT_{1A} receptor efficiency within the MBH, progesterone decreases the amount of 5-HT available to activate the inhibitory 5-HT_{1A} receptors.

Since 5-HT_{1B} receptors, located on terminals of 5-HT neurons, function as autoreceptors to control 5-HT release (Daws, et al., 2000; Engel, et al., 1986; Maura, et al., 1986; Riad, et al., 2000), these receptors might be expected to play a positive role in control of lordosis behavior. Furthermore, progesterone has been suggested to increase 5-HT_{1B} receptors in the MBH (Frankfurt, et al., 1994). A facilitatory role for 5-HT_{1B} receptors in the 5-HT nerve terminal region of the VMN has been suggested (Frankfurt et al., 1994; Mendelson, 1992; Mendelson and Gorzalka, 1986), and a variety of nonselective 5-HT_{1B} receptor agonists, but not all (Hunter, et al., 1985), can increase lordosis behavior (Aiello-Zaldivar, et al., 1992; Mendelson, 1992; Mendelson and Gorzalka, 1990). However, until recently, selective 5-HT_{1B} agents were not available for evaluation of this suggestion (Sari, 2004). In the following experiments, the 5-HT_{1B/1D} receptor antagonist, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1-1'-biphenyl-4-carboxamide hydrochloride (GR 127935), (Liao, et al., 2000) was employed. If rats, hormonally primed with estrogen and progesterone (EP rats), have enhanced functioning and/or density of 5-HT_{1B} receptors in the VMN, EP rats should be less sensitive to a 5-HT_{1B} receptor antagonist agonist than rats hormonally primed only with estrogen (EO rats). As reported below, this appears to be the case.

Additionally, experiments are described that were designed to test the hypothesis that progesterone's modulation of 5-HT_{1B} receptors contributes to the hormone's protection against a 5 min restraint stress. In previous studies, we reported that 5 min restraint transiently reduced lordosis behavior of EO rats but had no effect in EP rats (Truitt, et al., 2003; White and Uphouse, 2004). Since stress increases release of 5-HT in a variety of brain areas (Beekman, et al., 2005; Fujino, et al., 2002; Kirby, et al., 1997), including the MBH (Shimizu, et al., 1992), a progesterone-mediated enhancement of terminal 5-HT_{1B} receptor functioning could be involved in the EP rats' resistance to stress. It was, therefore, hypothesized that antagonism of 5-HT_{1B} receptor function would enhance the vulnerability of EP rats to this restraint. However, while GR 127935 did amplify the effect of restraint in EO rats, there was no such amplification in EP rats.

Methods

Materials

Estradiol benzoate, progesterone, sesame seed oil, and GR 127935 (5-HT_{1B/1D} receptor antagonist; N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1-1'-biphenyl-4-carboxamide hydrochloride) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Isoflurane (AErrane[®]) and suture material (vicryl) were purchased from Henry Schein (Melville, NY). Intracranial (i.c.) cannulae were obtained from Plastics One (Roanoke, VA) and dental acrylic was purchased from Reliance Dental (Worth, IL). Disposable Decapicone[®] restrainers were purchased from Braintree Scientific Inc. (Braintree, MA). All other supplies came from Fisher Scientific (Houston, TX).

Animals and housing

Female, adult Fischer (F-344) rats were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed 2 or 3 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 lib*. All procedures were conducted according to PHS policy and were approved by the IACUC at Texas Woman's University.

Surgical procedures and hormone treatments

Females, weighing between 140 and 170 g (approximately 60 to 90 days of age), were anesthetized with AErrane[®] and stereotactically implanted bilaterally with 22-gauge stainless steel cannulae directed toward the VMN [atlas coordinates AP 4.38; DV - 7.8; ML $\pm$ 0.4 (König and Klippel, 1963)] as previously described (Uphouse, et al., 1992). Two weeks later, rats were ovariectomized under AErrane[®] anesthesia (White and Uphouse, 2004) and injected two weeks later with 10 µg of estradiol benzoate followed 48 hr later with sesame seed oil or 500 µg progesterone. Hormones were dissolved in sesame seed oil and were injected subcutaneously (s.c.) in a volume of 0.1 ml/rat. Behavioral testing occurred 4 to 6 hr after progesterone or oil treatment.

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., 1992), was initiated within 1 to 3 hr after colony lights off and 4 to 6 hr after the progesterone or oil injection. Visibility was aided by red lighting. In a 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 lordosis to mount (L/M) ratio of 0.7 or higher were included in the remaining experiments. Lordosis quality and proceptivity, as previously described (Hardy and DeBold, 1971; White and Uphouse, 2004), were also recorded. Following the pretest, rats were infused with GR 127935 or water and treated as described in the specific experiments. GR 127935 was dissolved in distilled/deionized water immediately before use. Infusions were delivered at a rate of 0.24–0.26 µl/min to a final infusion volume of 0.5 µl per cannula. Intracranial drug concentrations are indicated as the amount of drug infused per bilateral cannula or one-half the concentration per animal. Sexual behavior testing continued for 30 consecutive min as previously described (Uphouse et al., 2003).

Specific experimental procedures

A total of 111 rats were used in three separate experiments. The number of rats used in the first, second and third experiments, respectively, were 26, 32 and 53. In the first experiment, rats were not specifically handled between ovariectomy and use in the experiment. EO or EP

rats were infused intracranially with water or 200 ng GR 127935 and tested immediately after infusion. In the second and third experiments, rats were specifically handled, as described below, prior to the day of infusion. In the second experiment, rats were infused and tested as in the first experiment. In the third experiment, rats were either restrained or remained in the home cage for 5 min following infusion and were tested immediately after the 5 min experience.

Histological procedures

After behavioral testing, rats were anesthetized with AErrane[®] and perfused with 0.9% saline followed by 10% buffered formalin. Brain tissue was placed into 10% buffered formalin for at least 24 h before sectioning. Coronal sections (100 μ m) were stained with cresyl violet and examined for cannulae placement (König and Klippel, 1963). Only data from rats with cannulae in the MBH in the vicinity of the VMN were included in the study.

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 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.

Restraint procedures

Restraint procedures were as previously described (Uphouse, et al., 2007). After infusion, 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. A longitudinal slit was made along the cone to allow room for the top of the guide cannulae. The slit was then secured with lab tape after the female's nose was flush with the air hole. The base of the cone was gathered around the female's tail and secured tightly with tape. The process of wrapping the female required between 30 and 60 sec. The wrapped female was set aside for 5 min. Nonrestrained rats were returned to their home cages for 5 min.

Statistical procedures

For statistical purposes, L/M ratios, lordosis quality scores and number of mounts by the male were grouped into the pretest interval and five-min intervals after infusion or restraint. Data were evaluated by repeated measures ANOVA with hormone, type of infusion and/or type of experience as the independent factors. Time after infusion (or experience in experiment 3) was the repeated factor. Differences between treatment groups, within time intervals, were compared with Tukey's test. Comparisons within groups, across time, were made with Dunnett's test to the pretest interval. An alpha level of 0.05 was required for rejection of the null hypothesis (Zar, 1999).

Results

Experiment 1

As expected, GR 127935 reduced lordosis behavior of EO, but not EP, rats (Figure 1A). However, infusion with the water vehicle also reduced sexual behavior of EO rats. There were significant effects of hormone treatment ($F_{1,22} = 25.6$, $P \leq 0.0003$), time after infusion ($F_{6,132} = 6.47$, $P \leq 0.0001$), and their interaction ($F_{6,132} = 4.88$, $P \leq 0.0002$). Infusion with GR 127935 led to greater decrements in the L/M ratio than did the water infusion ($F_{1,22} = 7.83$, $P \leq 0.02$) that was primarily evident in the EO rats (interaction between type of infusion and hormone, $F_{1,22} = 5.12$, $P \leq 0.04$). The water infusion reduced L/M ratios of EO rats at the 5 min test time (Dunnett's $q_{120,7} = 3.75$, $P \leq 0.05$) while infusion with GR 127935 reduced the

L/M ratios of EO rats at 5, 10, 20 and 25 min after infusion (Dunnett's $q_{120,7} \geq 2.60$, all $P \leq 0.05$). In contrast, neither the water nor the GR 127935 infusion reduced L/M ratios of EP rats. After water infusion, EO and EP rats differed significantly only at the 5 min test interval (Tukey's $q_{120,4} = 4.52$, $P \leq 0.05$), but after GR 127935 infusion, a hormonal effect was present at 5, 10, 20 and 25 min after the infusion (Tukey's $q_{120,4} \geq 3.685$, $P \leq 0.05$).

One EO rat infused with GR 127935 had an L/M ratio of zero during the testing and was, therefore, excluded from analysis of lordosis quality. For the remaining rats, lordosis quality was lower in EO rats than in EP rats ($F_{1,21} = 12.99$, $P \leq 0.002$; Figure 1B). However, this difference was primarily due to the effect of GR 127935 in EO rats leading to a significant effect of the type of infusion ($F_{1,21} = 13.70$, $P \leq 0.002$). None of the interactions terms were significant (all $P > 0.05$).

There was no significant effect of any of the treatments or their interactions on mounting behavior (all $P > 0.05$). For GR 127935 and water, respectively, in EO rats, the mean $\pm$ S.E. number of mounts per interval were 6.46 ± 0.75 and 5.28 ± 0.79 . For EP rats, the average mounts were 5.83 ± 0.43 and 6.25 ± 0.34 .

Experiment 2

The transient decrease in L/M ratios in EO rats infused with water was reminiscent of prior evidence that an acute 5 min stressor produced a transient decline in L/M ratios in EO, but not EP, rats (Truitt et al., 2003; White and Uphouse, 2004). Since rats had not been specifically handled between ovariectomy and testing, we considered the possibility that the infusion procedure, itself, was acting as an acute stressor in the implant EO rats and that GR 127935 had amplified this effect.

We, therefore, examined the effect of a water or GR 127935 infusion in ovariectomized rats that had been repeatedly handled (see Methods) prior to the experiment. There was a small, but significant, time-dependent decline in the L/M ratio ($F_{6,174} = 3.59$, $P \leq 0.05$) after infusion, but no main effects and none of the interaction terms were significant ($P > 0.05$). However, as can be seen in Figure 2, even in handled rats, EO rats continued to show a small response to the infusion. Similarly, none of the main effects were significant for lordosis quality (data not shown). However, there was a slight time-dependent decline in the number of mounts received ($F_{6,174} = 16.04$, $P \leq 0.05$) which was accentuated in EO rats (time $\times$ hormone interaction, $F_{6,174} = 2.91$, $P \leq 0.05$). The mean $\pm$ S.E. number of mounts per interval were 5.8 ± 0.34 and 5.33 ± 0.38 for EO GR 127935 and water groups, respectively. For EP rats, GR 127935 and water groups had 6.0 ± 0.49 and 6.51 ± 0.60 mounts per 5 min interval.

In a posthoc omnibus ANOVA, the L/M ratios from the first and second experiments were combined for analysis to assess the effect of handling. Although the main effect of handling was not significant, there was a significant interaction between hormone and handling ($F_{1,51} = 9.02$, $P \leq 0.005$) and a significant interaction between time and handling ($F_{6,306} = 2.72$, $P \leq 0.02$). This interaction reflected primarily higher L/M ratios of handled relative to nonhandled EO rats.

Experiment 3

The third experiment was designed to test the hypothesis that effects of restraint on L/M ratios would be accentuated by treatment with GR 127935. All rats were handled prior to the experiment. GR 127935 amplified the effects of restraint in EO but not in EP rats (Figure 3). There was a significant effect of hormone ($F_{1,45} = 33.78$, $P \leq 0.0001$), time ($F_{6,270} = 10.29$, $P \leq 0.0001$), time $\times$ type of infusion ($F_{6,270} = 3.84$, $P \leq 0.002$) and time $\times$ type of 5 min experience ($F_{6,270} = 2.32$, $P \leq 0.04$). Supporting the idea that the infusion procedure was mildly stressful

even in handled EO rats, both water and GR 127935 EO rats showed a decrease in their L/M ratio during the first 5 min after infusion (Dunnett's all $q \geq 2.57$, $P \leq 0.05$, Figure 3A). Restraint plus GR 127935 accentuated and prolonged this decrease, but EO water restraint and GR 127935 restraint rats differed significantly only at the 5 min interval ($q_{270,8} = 4.69$, $P \leq 0.05$). L/M ratios of EP rats were never significantly different from their respective pretest values (Figure 3B, all $P > 0.05$).

Coincident with the decline in L/M ratios, females became resistive to the male's attempts to mount and engaged in fighting, boxing and escape behaviors. Proceptivity was low throughout the experiment and did not differ across infusion or experience.

For 10 EO rats (5 EO drug and restraint, 3 EO drug no restraint, 2 EO water restraint, and 1 EO water no restraint), the L/M ratio fell to zero during the test so that lordosis quality could not be examined. For the remaining rats, lordosis quality was slightly lower in EO relative to EP rats ($F_{1,35} = 5.06$, $P \leq 0.04$) and lordosis quality declined during the test period ($F_{6,210} = 3.10$, $P \leq 0.007$) (data not shown). EO rats received slightly fewer mounts than EP rats ($F_{1,45} = 5.25$, $P \leq 0.03$; respectively mean $\pm$ S.E. for EO and EP rats = 6.08 ± 0.23 and 7.59 ± 0.34) and GR 127935 slightly reduced the number of mounts ($F_{1,45} = 4.10$, $P \leq 0.05$; respectively for GR 127935 and water, mean $\pm$ S.E. = 6.08 ± 0.26 and 7.34 ± 0.29).

Cannulae locations for rats infused with water or GR 127935 and then restrained for 5 min are shown in Figure 4. Nonrestrained rats had cannulae located in comparable positions.

Three additional EP rats were infused with 1000 ng GR 127935 and restrained for 5 min. Even with this high dose of the drug, the L/M ratio did not decline ($P > 0.05$).

Discussion

There were two major objectives of the current studies: (1) to determine if EO rats were more vulnerable to the lordosis-inhibiting effect of a 5-HT_{1B} receptor antagonist than EP rats and (2) to determine if the 5-HT_{1B} receptor antagonist would eliminate the resistance of EP rats to the lordosis-inhibiting effect of 5 min restraint. Although GR 127935 can act as a partial agonist under some conditions, it is primarily recognized as a selective 5-HT_{1B/1D} receptor antagonist (Beer, et al., 1998; de Groote, et al., 2003). Nevertheless, further study will be required to confirm that the effects of GR 127935, seen in the current experiments, result from an antagonist action of the drug.

Previously, we have shown that 5 min restraint transiently inhibits female rat lordosis behavior of EO, but not EP, rats (Truitt et al., 2003; White and Uphouse, 2004). Since stress increases extracellular 5-HT (Beekman et al., 2005; Fujino et al., 2002; Kirby et al., 1997; Lanfumey, et al., 2008; Shimizu et al., 1992) and 5-HT infusion into the VMN inhibits lordosis behavior (Foreman and Moss, 1978; Uphouse, et al., 1993), we hypothesized that the inhibition present in EO rats resulted from a stress-induced increase in release of 5-HT leading to activation of inhibitory 5-HT_{1A} receptors in the VMN. We reasoned that terminal 5-HT_{1B} receptors, if enhanced by progesterone, should reduce the stress-induced elevation of 5-HT and thereby attenuate the lordosis inhibition. If so, then EP rats should be less sensitive to the effects of a 5-HT_{1B} receptor antagonist. This appeared to be the case. A greater sensitivity of EO than EP rats to stress has been previously documented (Figueiredo, et al., 2002; Truitt et al., 2003) and 5-HT_{1B} receptors have been implicated in modulating the response to stress (Clark and Neumaier, 2001; Clark, et al., 2004). GR 127935's amplification of effects of stress in EO rats is consistent with the greater stress responsiveness of EO rats and with prior findings that corticosterone treatment increased a GR 127935-induced elevation in extracellular 5-HT in the rat hypothalamus (Gur, et al., 2001). However, the effects of restraint were only amplified in EO rats, and not EP rats, infused with the 5-HT_{1B} receptor antagonist. Moreover, an unexpected

finding in the current studies was the effect of the water vehicle infusion in EO rats. This apparent infusion stress was also amplified by GR 127935 in nonhandled EO rats, but not in handled, EO rats. The similarity between the effects of 5 min restraint and effects of the infusion allow the suggestion that the infusion was also a stressor and that in EO rats, a 5-HT_{1B} receptor antagonist enhanced the response to stress.

While these findings are consistent with prior suggestions that 5-HT_{1B} receptors may contribute to the facilitation of lordosis behavior of EO rats (Mendelson, 1992), 5-HT_{1B} receptors appear to be less important in EP rats. If an amplification of 5-HT_{1B} receptors were responsible for progesterone's attenuation of the effects of stress, EP rats infused with the 5-HT_{1B} receptor antagonist should behave like EO rats after the 5 min restraint. This did not appear to be the case. Therefore, progesterone's protection against restraint stress must include mechanisms in addition to an increase in 5-HT_{1B} receptors. A concentration of GR 127935 that was 5-fold higher than required to inhibit lordosis behavior of EO rats was ineffective in EP rats. Either 5-HT_{1B} receptors of EP rats remained unsaturated even with the highest concentration of GR 127935 or 5-HT_{1B} receptors, alone, are not responsible for the different vulnerabilities of EO and EP rats to the 5 min restraint.

Progesterone's ability to facilitate lordosis behavior includes effects mediated through intracellular progesterone receptors and through progesterone metabolites, such as 3 alpha-hydroxy-5alpha-pregnan-20-one (allopregnanolone) (Frye, 2001; Frye and Vongher, 2001). Progesterone metabolites have anxiolytic actions and have been reported to reduce the response to stress (Frye, 2007; Pinna, et al., 2008). Since brain concentrations of allopregnanolone are higher in EP than in EO animals (Frye, et al., 1998), it is possible that elevations in progesterone metabolites in EP rats compensated for GR 127935 blockade of 5-HT_{1B} receptors. In addition to their role as terminal autoreceptors, 5-HT_{1B} receptors also function as heteroreceptors on terminals of nonserotonergic neurons (Barnes and Sharp, 1999; Bramley, et al., 2005; Fink and Gothert, 2007; Sari, 2004). 5-HT_{1B} heteroreceptors and progesterone metabolites could provide redundant control of neurotransmitters, in addition to 5-HT, that reduce lordosis behavior. If so, antagonism of 5-HT_{1B} receptors would have minimal effectiveness in the presence of such progesterone metabolites. It would be interesting to test this hypothesis in EP rats in the presence of an inhibitor of 5-alpha reductase to reduce accumulation of the progesterone metabolites.

Therefore, while the rationale for these studies was based on the recognized role that terminal 5-HT_{1B} receptors play in controlling 5-HT release (Daws et al., 2000; Engel et al., 1986; Maura et al., 1986; Riad et al., 2000), the findings lead us to believe that receptors other than (or in addition to) terminal 5-HT_{1B} autoreceptors contribute to the observed differences between EO and EP rats. In addition, we cannot rule out a possible role of 5-HT_{1D} receptors in the response to GR 127935.

Finally, although GR 127935 is recognized as a relatively selective 5-HT_{1B/1D} receptor antagonist, there is evidence that it can act as a partial agonist under some conditions (Beer, et al., 1998; de Groote, et al., 2003). Therefore, additional experiments will be required to define the precise role of 5-HT_{1B} receptors in modulation of lordosis behavior of EO rats. Nevertheless, these experiments are important in expanding the understanding of serotonin's role in modulation of female rat lordosis behavior and may implicate VMN 5-HT_{1B/1D} receptors as one of the components involved in the effects of progesterone.

In summary, 5-HT_{1B/1D} receptors may be important in preventing lordosis inhibition but are not required for maintenance of the behavior in a rat with optimal hormone (estrogen and progesterone) priming. This dichotomy between effects of a 5-HT receptor antagonist in suboptimally hormone primed and maximally primed rats has also been reported for 5-HT₂

receptors (Uphouse, 2000) and underscores the different vulnerability of EO and EP rats to lordosis disruption.

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Figure 1. Infusion and GR 127935 effects in nonhandled, hormonally primed, ovariectomized rats Ovariectomized rats with bilateral cannulae near the VMN were hormonally primed with 10 μ g estradiol benzoate and oil (EO) or with estradiol benzoate and 500 μ g progesterone (EP). After a pretest (PRE) for sexual receptivity, rats were infused with 200 ng GR 127935 or the water vehicle. Lordosis behavior was monitored for 30 consecutive min thereafter. Data are the mean $\pm$ S.E. L/M ratios (1A) or lordosis quality (1B) for the pretest and 5 min intervals after infusion. N's for EO rats infused with water or GR 127935, respectively, were 8 and 5; for EP rats, N's were 7 and 6. @ indicates difference from the pretest interval; * indicates effect of hormone within drug and time; ** indicates effect of GR 127935 within hormone and time.

Figure 2. Infusion and GR 127935 effects in handled, hormonally primed, ovariectomized rats Ovariectomized rats with bilateral cannulae near the VMN were hormonally primed with 10 μ g estradiol benzoate and oil (EO) or with estradiol benzoate and 500 μ g progesterone (EP). Beginning with the day of estrogen priming, rats were handled for two consecutive days as described in the Methods. Behavioral testing proceeded as described in Figure 1. Data are the mean $\pm$ S.E. L/M ratios for the pretest (PRE) and 5 min intervals after infusion. N's for EO rats infused with water or GR 127935, respectively, were 12 and 5; for EP rats, N's were 10 and 6.

Figure 3. Effects of water or GR 127935 infusion in restrained rats

Ovariectomized rats with bilateral cannulae near the VMN were hormonally primed with 10 μ g estradiol benzoate and oil (EO) or with estradiol benzoate and 500 μ g progesterone (EP). Beginning with the day of estrogen priming, rats were handled for two consecutive days as described in the Methods. After the pretest (PRE) for sexual receptivity, rats were infused with 200 ng GR 127935 or the water vehicle. Rats were then restrained for 5 min or returned to their home cage for 5 min. Lordosis behavior was monitored for 30 consecutive min thereafter. Data for EO rats are shown in 3A; data for EP rats are shown in 3B. Data are the mean $\pm$ S.E. L/M ratios for the pretest and 5 min intervals after the restraint or home cage experience. For EO rats, N's for water home cage, water restraint, GR 127935 home cage, and GR 127935 restraint,

respectively, were 7, 9, 5, and 7. For EP rats, N's were 7, 7, 6, and 5, respectively. @ indicates difference from the pretest interval; * indicates difference from EO water and GR 127935 restraint.

Figure 4. Cannulae locations for restrained rats

In the figure are shown cannulae locations for restrained rats used in the third experiment. Coronal sections are taken from König and Klippel (1963). Cannula location was assigned to the brain section containing the most ventral location of the cannula track. Filled circles on each section indicate bilateral locations from rats that were infused with 200 ng GR 127935. Open circles indicate cannulae locations for rats infused with the water vehicle. Sites located in EO rats are shown on the left and those for EP rats are shown on the right.