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Progesterone reduces the inhibitory effect of a serotonin 1B receptor agonist on lordosis behavior

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ABSTRACT

Ovariectomized Fischer inbred rats were hormonally primed with 10 μg estradiol benzoate and sesame seed oil (EO rats) or with estradiol benzoate and 500 μg progesterone (EP rats). Four to six hours after progesterone or oil, rats were pretested for sexual behavior and then infused bilaterally into the ventromedial nucleus of the hypothalamus with 0, 50, 100 or 200 ng of the 5-HT_{1B} receptor agonist, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-bi]pyridin-5-one-dihydrochloride (CP 93129). Sexual receptivity was monitored by the lordosis to mount (L/M) ratio. EO rats showed a transient decline in lordosis behavior following infusion with the saline vehicle and this was amplified by CP 93129. There were no effects of any infusion in EP rats. These findings are discussed in terms of the possible stress effect of the intracranial infusion in EO rats and their implications for a role of 5-HT_{1B} receptors in the response to a mild stress.

1. Introduction

Lordosis behavior is a sexually receptive posture made by female rats in response to mounting by a male. In naturally cycling females, the gonadal hormones, estradiol and progesterone, synchronize the behavior with ovulatory events so that successful reproduction can occur (Blaustein, 2008; Pfaff et al., 2008). The lordosis reflex (e.g. arching of the back in response to the male's mount) requires estradiol while progesterone enhances the probability that the reflex will occur and reduces the dose of estradiol that is required to facilitate the behavior (Blaustein, 2008; Sodersten, 1981). In addition, behaviors designed to attract the attention of the male (e.g. proceptive and solicitous behaviors) are enhanced by progesterone (Erskine, 1989).

When ovariectomized Fischer female rats are hormonally primed with 10 μg estradiol benzoate, approximately 80% of the female show high levels of lordosis responding (e.g. females exhibit the reflex almost every time the male mounts) (Truitt et al., 2003; White and Uphouse, 2004). However, relative to females primed with estradiol and progesterone (EP rats), females that are primed only with estradiol (EO rats) are more likely to exhibit resistive behavior toward the male's attempts to mount and are less likely to be solicitous toward the male. Fischer rats have been reported to show evidence of high emotionality in a variety of behavioral tests (Dhabhar et al., 1993; Izumi et al., 1996; Rosecrans et al., 1986; Stohr et al., 2000) and show a larger hypothalamic–pituitary–adrenal response to stress than several other rat strains (Armario et al., 1995; Dhabhar et al., 1995; Gomez-Serrano et al., 2009; Marissal-Arvy et al., 2007; Rosecrans et al., 1986; Sarrieau and Mormede, 1998). Lordosis behavior of estradiol benzoate-primed Fischer females is also vulnerable to disruption by a mild stress (Truitt et al., 2003; Uphouse et al., 2009; White and Uphouse, 2004). In prior experiments, we have shown that a stressor as mild as 5 min of restraint can substantially reduce lordosis behavior of EO Fischer rats while having no effect in EP rats (Truitt et al., 2003). Fischer rats may, therefore, be especially vulnerable to stress-induced declines in lordosis behavior and/or particularly sensitive to the anxiolytic effects of progesterone. However, in EP rats, lordosis inhibition after mild restraint was present when rats were pretreated with a 5-HT_{2C} receptor antagonist (Uphouse et al., 2003); and the lordosis-inhibitory effects of a 5-HT_{1A} receptor agonist were increased by the restraint (Uphouse et al., 2003). These findings led us to speculate that progesterone's protection against the effects of mild restraint includes modified functioning of the serotonin system. In support of this speculation, a 5-$HT_{2A/2C}$ receptor agonist attenuated the restraint-induced lordosis inhibition in EO Fischer rats (Uphouse et al., 2007).

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Serotonin modulates a variety of behaviors, including sexual behavior (Blundell et al., 1995; Jonnakuty and Gragnoli, 2008; Mendelson, 1992; Uphouse, 2000); and extracellular 5-HT (as measured by microdialysis procedures) increases in a variety of brain areas in response to stressors (Beekman et al., 2005; Fujino et al., 2002; Kirby et al., 1997; Mo et al., 2008; Shimizu et al., 1992). In the mediobasal hypothalamus, extracellular serotonin declines coincident with the emergence of sexual receptivity (e.g. lordosis behavior) (Maswood et al., 1999) and progesterone appears to be responsible for this decline (Farmer et al., 1996; Maswood et al., 1999). These observations allow the hypothesis that progesterone's reduction of extracellular 5-HT may contribute to its protection against the effects of mild stress on lordosis behavior.

Although primarily inhibitory to lordosis behavior, serotonin's precise role depends on the receptor subtype activated (Mendelson, 1992; Uphouse, 2000). For example, in the ventromedial nucleus of the hypothalamus (VMN), agonist activation of 5-HT_{1A} receptors inhibits the behavior while agonist activation of 5-HT_2 receptors can facilitate the behavior (Uphouse, 2000). Estrogens and progesterone influence the serotonergic system (Bethea et al., 2002) and modulate the effects of agonists at 5-HT_{1A} and 5-HT_2 receptor subtypes (Jackson and Uphouse, 1998; Sinclair-Worley and Uphouse, 2004). While the differential roles of 5-HT_{1A} and 5-HT_2 receptors in serotonin's modulation of lordosis behavior are well established, the contribution of other receptor subtypes has received less investigation. Two different laboratories have reported data consistent with a positive influence of 5-HT_3 receptors on lordosis (Maswood et al., 1997, 1998; Tanco et al., 1993), and there is evidence that activation of 5-HT_7 receptors may inhibit the behavior (Siddiqui et al., 2004; Siddiqui et al., 2007). In early studies with nonselective 5-HT_{1B} receptor compounds, a facilitative role for 5-HT_{1B} receptors was suggested (Aiello-Zaldivar et al., 1992; Mendelson and Gorzalka, 1990). In support of this possibility, infusion of a selective 5-HT_{1B} receptor antagonist, GR 127935, into the VMN amplified the negative effects of 5 min restraint on lordosis behavior of estradiol benzoate-primed ovariectomized rats (Uphouse et al., 2009).

However, an unexpected finding emerged from the study with GR 127935 (Uphouse et al., 2009). Specifically, when rats primed only with estradiol benzoate received vehicle infusions into the VMN, a transient decline in lordosis behavior was observed that was never present in EP rats (Uphouse et al., 2009). The magnitude of this decline depended on the rats' previous experience so that prior handling reduced, but did not eliminate, the effect of the infusion; and in nonhandled rats, GR 127935 amplified the effects of the infusion. These experiments led to the conclusion that the overall process of the intracranial infusion, itself, was mildly stressful to the EO rats and that GR 127935 amplified the lordosis-inhibitory effects of the stress. Since 5-HT_{1B} receptors can function as terminal autoreceptors leading to a reduction in 5-HT release (Auerbach et al., 1991; Engel et al., 1986; Hjorth and Tao, 1991), 5-HT_{1B} receptor agonists might be expected to facilitate lordosis behavior by reducing extracellular 5-HT.

If this hypothesis is correct, then a 5-HT_{1B} receptor agonist might be expected to reduce the effects of the intracranial infusion process on lordosis behavior of EO rats. The following experiment was designed to test this hypothesis. Because of our past experience with this brain area, the VMN was selected for the infusion location. The objective of the study, however, was to further explore the effects of the infusion "stress" and not to identify the specific brain area(s) responsible for the effects of the experimental manipulations.

2. Materials and methods

2.1. Materials

Estradiol benzoate, progesterone, and sesame seed oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The 5-HT_{1B} receptor agonist, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-bi]pyridin-5-one-dihydrochloride (CP 93129), was purchased from Tocris Bioscience (Ellsville, MO). Isoflurane (AErrane®) and suture material (vicryl) were purchased from Henry Schein (Melville, NY). Intracranial (i.c.) cannulae and dental acrylic were obtained, respectively, from Plastics One (Roanoke, VA) and Reliance Dental (Worth, IL). Decapicone® restrainers were from Braintree Scientific, Inc. (Braintree, MA). Other supplies came from Fisher Scientific (Houston, TX).

2.2. Animals and housing

Adult Fischer (F-344) female rats, purchased from Charles River Laboratories (Wilmington, MA), 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.

2.3. Surgical procedures and hormone treatments

When approximately 60 to 90 days of age, females 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 ± 0.4 (König and Klippel, 1963)] as previously described (Uphouse et al., 1992). No neuroanatomical controls were included for evaluation of the specificity of the infusion effects. Two weeks after implant surgery, rats were ovariectomized under AErrane® anesthesia (White and Uphouse, 2004). Two weeks after ovariectomy, rats were injected with 10 μg of estradiol benzoate followed 48 h later with 500 μg progesterone (for EP rats) or the sesame seed oil vehicle (for EO rats). Hormones, dissolved in sesame seed oil, were injected subcutaneously (s.c.) in a volume of 0.1 ml/rat. Behavioral testing occurred 4 to 6 h after progesterone or oil treatment.

2.4. 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 h after colony lights off and 4 to 6 h after the progesterone or oil injection. Experimenter visibility was aided by red lighting. In a pretest for sexual receptivity, females were placed into the home cages of sexually experienced Sprague–Dawley male rats and behavior was monitored for 10 min or 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 (hopping and darting), as previously described (White and Uphouse, 2004), were also recorded. Based on the presence or absence of proceptive and resistive behavior, females were categorized as showing or not showing the respective behaviors. Following the pretest, rats were infused bilaterally with 0.5 μl containing 50, 100, or 200 ng CP 93129 (in 9% saline) or the saline vehicle. Infusions were delivered at a rate of 0.24–0.26 μl/min. The cannulae remained in place for 2 min following completion of infusion. Immediately, thereafter, the female was returned to the male's cage for behavioral testing.

Intracranial drug concentrations are given as the amount of drug infused per bilateral cannula or one-half the concentration per animal. Sexual behavior testing continued for 30 consecutive minutes after infusion.

2.5. 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 μm) were stained with cresyl violet and examined for cannulae placement (König and Klippel, 1963). A total of 74 rats were used. Of these, 59 rats had cannulae located in the vicinity of the VMN. Fifteen rats, with cannulae outside the VMN, were excluded from the primary analysis. Rats with one cannula in the VMN and the second cannula outside the VMN were also excluded from the analysis.

2.6. Handling procedures

Handling began on the day of the estradiol benzoate injection as previously described (Uphouse et al., 2009). Each rat was picked up, held for 15 s, and turned as in preparation for an intraperitoneal injection. This procedure was then repeated and constituted a handling session. The procedure was repeated until each rat had received 5 handling sessions. The entire process was repeated the following day.

2.7. Statistical procedures

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. Data were evaluated by repeated measures ANOVA with hormone and type of infusion as the independent factors. Time relative to infusion 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. Proceptive and resistive behavior were compared with Chi-Square procedures. Statistical analyses were conducted with SPSS 17 or SuperAnova 1.11 for MacIntosh. Independent pair-wise comparisons were performed manually (Zar, 1999). An alpha level of 0.05 was required for rejection of the null hypothesis.

3. Results

Cannulae locations for rats used in the experiments are shown in Fig. 1 and L/M ratios are shown in Fig. 2. In agreement with a prior study (Uphouse et al., 2009), the saline infusion into the VMN reduced lordosis behavior of EO but not EP rats (Fig. 2). After saline infusion, L/M ratios of EO rats declined rapidly and were significantly different from their pretest at 10 and 15 min after the infusion (Dunnett's q (306,7) = 2.75 and 2.76, respectively; $P \leq 0.05$). Following VMN infusion of the 2 lowest doses of CP 93129 (50 and 100 ng), L/M ratios of EO rats were initially similar to, or even higher, than that of rats infused with saline. However, L/M ratios of saline-treated rats recovered from the lordosis inhibition while rats infused with either 50 or 100 ng CP 93129 did not. As a consequence, by 30 min after VMN infusion, L/M ratios of saline-treated rats neared pretest levels while that of 50 and 100 ng CP 93129-infused rats remained low and were significantly different from the saline group (Tukey's q (306,4) = 4.59 and 4.02, respectively, $P \leq 0.05$). Bilateral VMN infusion of 200 ng CP 93129 produced robust inhibition of lordosis behavior of EO rats at every test interval after infusion (Dunnett's all q (7306) ≥ 3.49, $P \leq 0.05$).

None of the treatments affected L/M ratios of EP-treated rats. Overall, EP rats had higher L/M ratios than EO rats (ANOVA for hormone priming, F (1,51) = 37.16, $P \leq 0.0001$); rats infused with 200 ng CP 93129 had the lowest L/M ratios (ANOVA for drug, F (3,51) = 2.97, $P \leq 0.04$) and this was most evident in EO-treated rats (ANOVA for hormone by drug interaction, F (3,45) = 3.10, $P \leq 0.04$). L/M ratios declined after VMN infusion (ANOVA for time, F (6,306) = 12.52, $P \leq 0.0001$) and this was also most evident in EO rats (ANOVA for interaction between time and type of hormonal priming, F (6,306) = 2.56, $P \leq 0.02$). The three-way interaction was not significant.

For analysis of lordosis quality, 8 rats were excluded (for EO rats, 1 each from saline, 50 and 100 CP 93129 conditions and 3 rats infused with 200 ng CP 93129; for EP rats, 1 rat infused with 100 ng CP 93129) because L/M ratios fell to zero during the testing period. For remaining rats, overall lordosis quality was slightly higher for EP than for EO rats (ANOVA for hormone, F (1,45) = 8.09, $P \leq 0.007$) (see Fig. 3). Although there was an effect of VMN infusion on lordosis quality (F (3,45) = 3.35, $P \leq 0.03$), this resulted primarily from the effect of 100 ng CP 93129 in EO rats (ANOVA for drug by hormone interaction, F (3,45) = 4.15, $P \leq 0.02$). There was also a significant effect of time (F (6,270) = 2.59, $P \leq 0.02$) as well as a time by drug interaction (F (18,270) = 1.83, $P \leq 0.01$). The three-way interaction was not significant and none of the posthoc pair-wise comparisons within time interval were significant (all $P > 0.05$).

Ovariectomized rats primed only with estradiol benzoate showed little proceptivity. However, 8 of the 25 EO-treated rats showed some evidence of proceptive and/or solicitous behavior during the pretest and, in EO rats, this was also eliminated by the VMN infusion (see Table 1). A majority of EP-treated rats showed proceptivity during the pretest and this was also reduced by CP 93129 (though not significantly so; Chi-Square = 1.50, df = 3, $P > 0.05$), but in a reverse dose-dependent manner. Rats also showed an increase in resistance after VMN infusion, but this occurred independently of the kind of infusion (Table 1).

Although neuroanatomical localization was not an objective of this experiment, every rat that was excluded from the primary data analysis was examined. The stress resulting from the process of infusion in EO rats did not appear to be dependent on the location of the cannulae (see Fig. 4). Of 8 saline-infused EO rats, 4 rats had cannulae located in the third ventricle or were lateral or dorsal to the VMN. Four other rats had one cannula in the VMN and the second cannula outside the VMN. A single rat (with both cannulae in the third ventricle) failed to show a decline in lordosis behavior after the infusion process. All 8 rats were pooled for data presentation in Fig. 4 and were compared by repeated measures ANOVA to saline-treated rats with both cannulae in the VMN. There were no significant differences between the two groups (F (1,11) = 1.44, $p > 0.05$).

One EO rat infused with 50 ng CP 93129 had both cannulae in the third ventricle and one rat had both cannulae in the dorsal area. Three EO rats with imperfect implants were infused with 100 ng CP 93129.

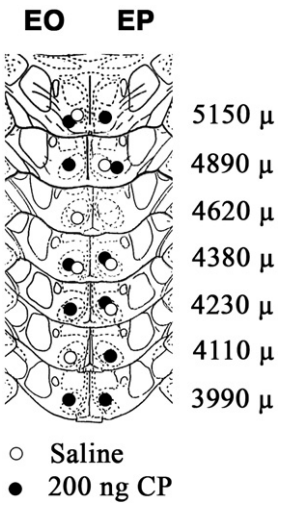

Fig. 1. Cannulae locations for rats infused with 200 ng CP 93129. Closed and open circles represent, respectively, cannulae locations for rats infused with 200 ng CP 93129 or saline. Rats were hormonally primed with 10 μg estradiol benzoate and sesame seed oil (EO) or 500 μg progesterone (EP). EO rats are represented on the left; EP rats are represented on the right. The numbers to the right of the sections represent atlas locations through the medial basal hypothalamus (König and Klippel, 1963).

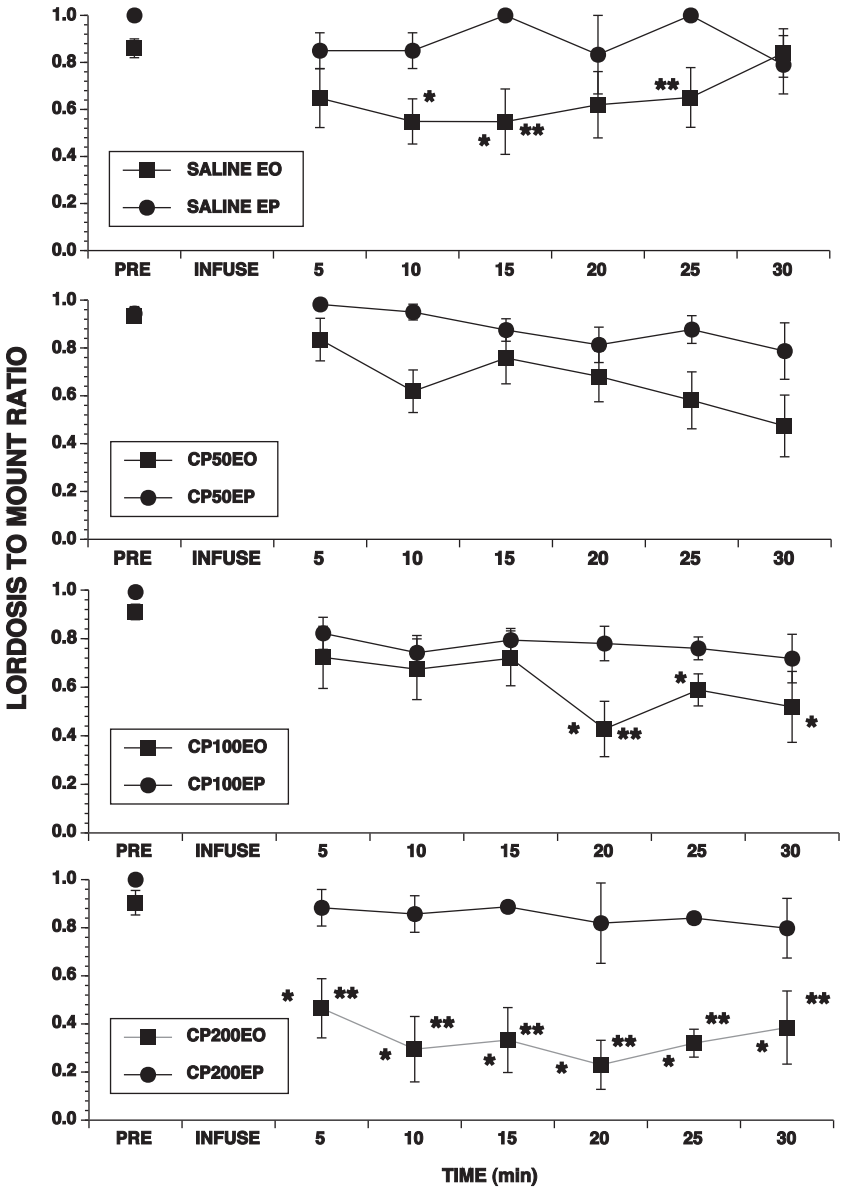

Fig. 2. L/M ratios of rats infused with CP 93129. Data are the mean ± S.E. L/M ratios of rats hormonally primed with 10 μg estradiol benzoate and sesame seed oil (EO) or 500 μg progesterone (EP) before (PRE) and after infusion with CP 93129. Postinfusion data are grouped into 5 min intervals. N's for saline and 50, 100, and 200 ng CP 93129, respectively, for EP rats are 3, 7, 12, and 12; for EO rats, 5, 8, 7, and 5. When S.E. is not visible, the magnitude was smaller than the size of the symbol. Single asterisks indicate a significant difference from the pretest. Double asterisks indicate time points when L/M ratios of EO rats were significantly different from those of EP rats.

One of these had both cannulae in the third ventricle; one had both cannulae dorsal to the VMN; and one had one cannula in the ventricle and one cannula in the VMN. One EO rat, infused with 200 ng CP 93129, had both cannulae located dorsal to the VMN. All these rats showed a decline in lordosis behavior characteristic of the process of saline infusion, but there were not a sufficient number of animals to compare to rats with the drug infusion in the target area.

4. Discussion

The primary objective of this experiment was to test the hypothesis that infusion of a 5-HT_{1B} agonist would reduce the effect associated with intra-VMN infusion in rats primed only with estradiol benzoate. Since activation of terminal 5-HT_{1B} autoreceptors reduces the release of 5-HT (Barnes and Sharp, 1999; Hannon and Hoyer, 2008; Hjorth and Tao, 1991), CP 93129 was expected to reduce any stress-induced, 5-HT-mediated inhibition of lordosis behavior.

In contrast to our expectations, CP 93129, infused into the VMN of EO rats, did not prevent the lordosis inhibition that resulted from the process of infusion; and the highest dose of CP 93129 produced robust and long-lasting inhibition of the lordosis behavior. This was especially surprising given prior findings with a 5-HT_{1B} receptor antagonist (Uphouse et al., 2009). However, although 5-HT_{1B} receptors located on 5-HT terminals are efficient negative regulators of 5-HT release, 5-HT_{1B} receptors also regulate a variety of other neurotransmitter systems, including GABA, glutamate, and dopamine (Alex and Pehek, 2007; Hannon and Hoyer, 2008), all of which can influence female rat lordosis behavior (Georgescu and Pfaus, 2006; Guptarak et al., 2004; Mani et al., 1996; McCarthy et al., 1995). CP 93129 activation of these heteroceptors may have been responsible for its failure to prevent lordosis inhibition. However, it is also possible that CP 93129 failed to reduce the effects of the infusion stress because the stress associated with the process of infusion occurred before the drug could be infused into the VMN. This would be

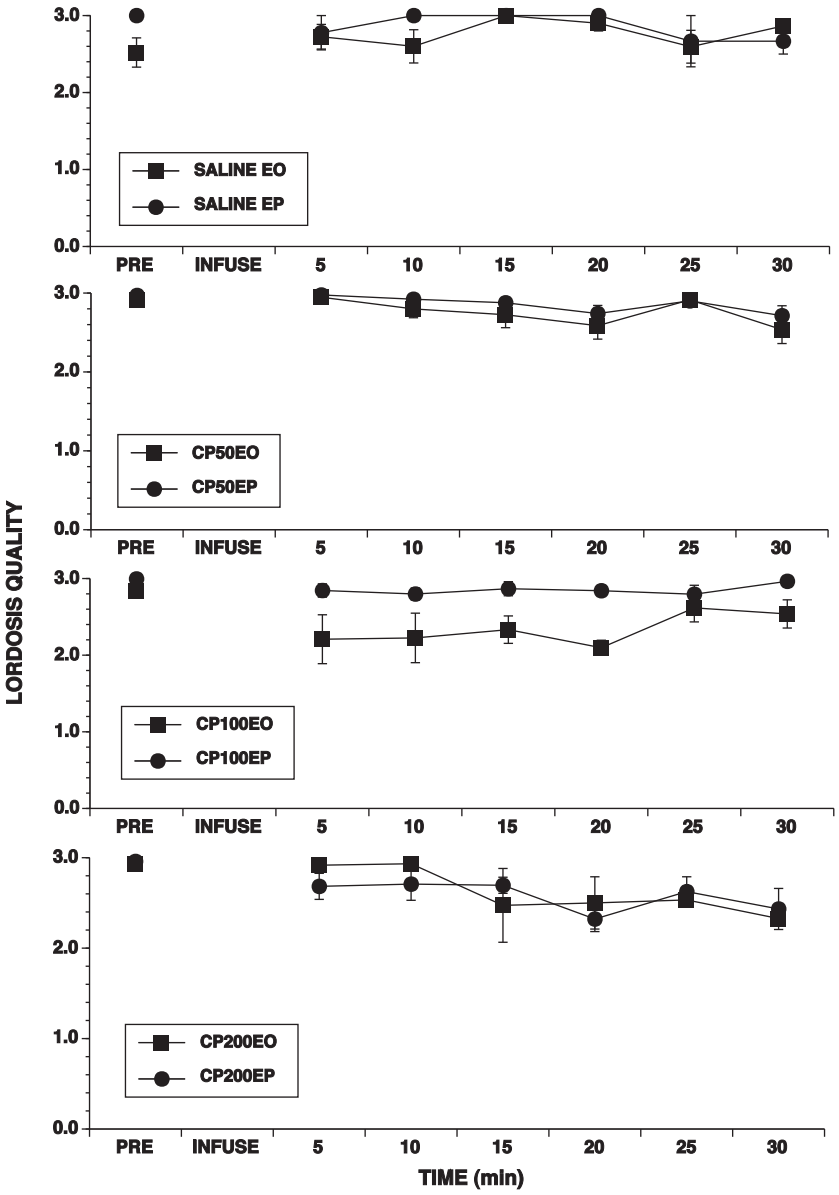

Fig. 3. Quality scores of rats infused with CP 93129. Data are the mean ± S.E. lordosis quality scores for rats hormonally primed with 10 μg estradiol benzoate and sesame seed oil (EO) or 500 μg progesterone (EP) before (PRE) and after infusion with CP 93129. When S.E. is not visible, the magnitude was smaller than the size of the symbol. Postinfusion data are grouped into 5 min intervals. N's for saline and 50, 100, and 200 ng CP 93129, respectively, for EP rats are 3, 7, 11, and 12; for EO rats, N's are 4, 7, 6, and 3.

consistent with the observation that the decline in lordosis behavior occurred independent of the infusion site. In either case, CP 93129 was not able to overcome this inhibition.

Although intracranial infusion of 5-HT to the medial basal hypothalamus inhibits lordosis behavior (Foreman and Moss, 1978; Mendelson, 1992), a rapid inhibition of the behavior is followed by a delayed recovery to preinfusion levels (Maswood et al., 1996). The inhibition and recovery appear to be mediated, respectively, by 5-HT_{1A} and 5-HT_2 receptors (Uphouse, 2000). The transient effect of the VMN saline infusion in EO rats mimicked this effect of 5-HT in that lordosis behavior declined rapidly but returned toward pretest levels within the 30 min test period. However, the decrease in lordosis behavior following the infusion process does not appear to require infusion into the VMN. Of 8 rats with at least one cannula residing outside the VMN, 7 showed a decline in L/M ratios following infusion with saline.

Following infusion of the 2 lowest doses of CP 93129 into the VMN, the initial inhibition of lordosis behavior was comparable to that of the saline infusion, but recovery failed to occur. With the highest dose of CP 93129, lordosis inhibition was robust and continued throughout the testing period. If, as previously suggested (Uphouse,

Table 1
Effect of VMN infusions on proceptive and resistive behavior.[a]

Hormone	Treatment	N	Proceptivity (%)	Resistance (%)
EO rats	Pretest	25	32	36
	Saline	5	0	80
	50 ng CP	8	0	88
	100 ng CP	7	0	58
	200 ng CP	5	0	60
EP rats	Pretest	34	74	6
	Saline	3	66	66
	50 ng CP	7	28.5	86
	100 ng CP	12	41.6	84
	200 ng CP	12	50	75

[a] Data are the percentage of rats showing the behavior before infusion (pretest) and during each of the infusion conditions listed.

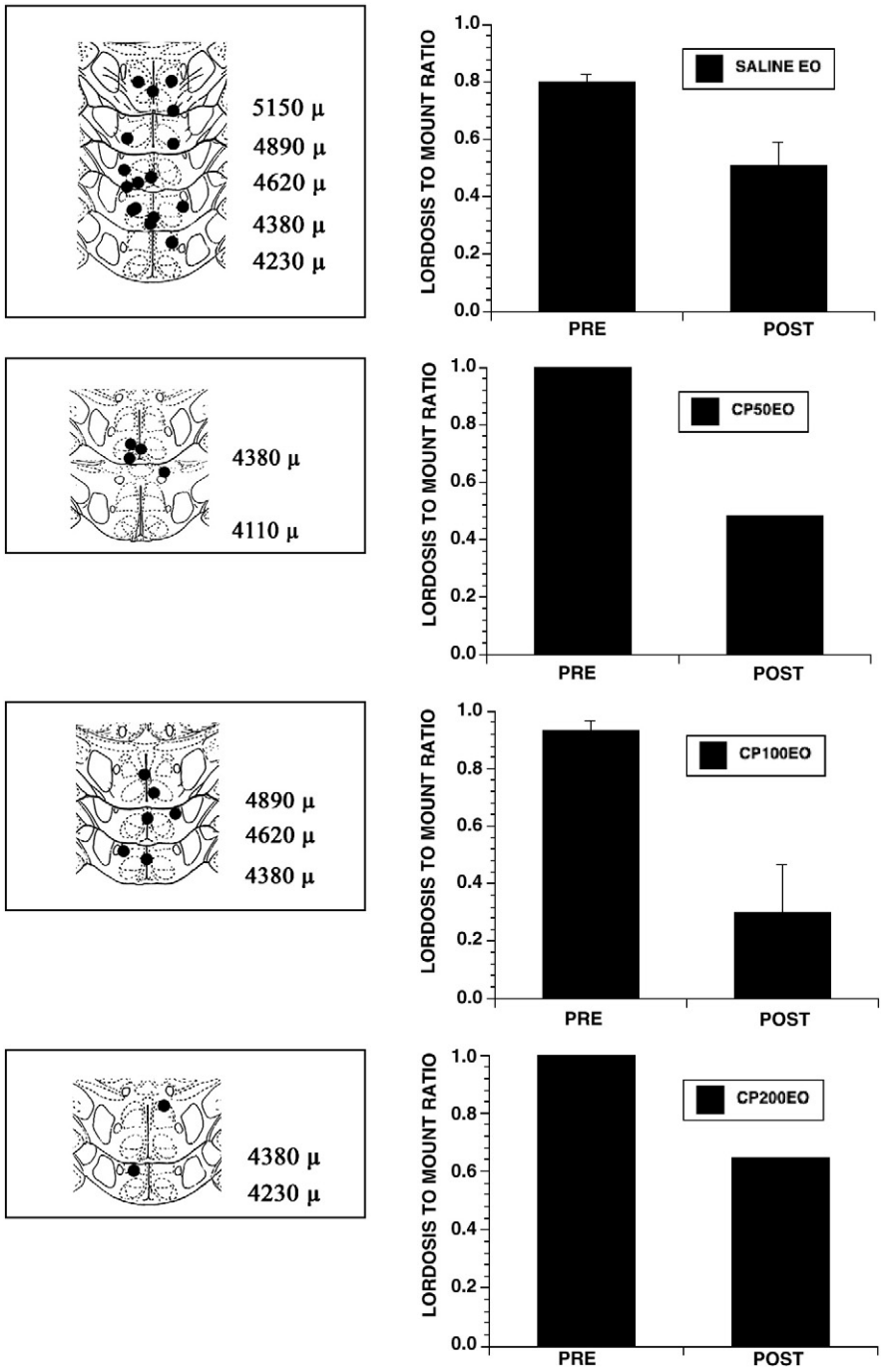

Fig. 4. L/M ratios for rats with cannulae implanted outside the VMN. Shown in the figure are the bilateral cannulae locations (on the left) and lordosis behavior (on the right) for EO rats having at least one cannula located outside the VMN. N's for saline and 50, 100 and 200 ng CP 93129 are 8, 2, 3, and 1. Data are the L/M ratios for the pretest before infusion (PRE) and the average L/M ratio/test interval during the 30 min test after infusion (POST). S.E. are shown only for rats infused with saline (n = 8) and 100 ng CP 93129 (n = 3).

2000), continued and/or high extracellular concentrations of 5-HT are required to activate the 5-HT_2 receptors responsible for the recovery process, CP 93129, by activation of terminal 5-HT_{1B} autoreceptors, might be expected to prevent extracellular accumulation of 5-HT in the VMN and thus prevent this recovery from taking place.

This possibility is especially interesting given results of a prior study with the 5-HT_{1B} receptor antagonist, GR 127935 (Uphouse et al., 2009). When rats were prehandled and received VMN infusions as in the current experiment, rats infused with GR 127935 did not differ significantly from the vehicle infusion. However, if VMN infusion with GR 127935 occurred before a 5 min restraint stress, GR 127935 amplified the initial negative effects of the restraint stress, as expected, but did not prevent recovery from taking place. Therefore, the current findings allow the suggestion that endogenous 5-HT released in the VMN in response to stress contributes to a stress-induced decline in the behavior but may also be important in limiting the duration of stress-induced lordosis inhibition. However, in neither the study with GR 127935 nor the current study was the experiment designed to identify specific brain areas responsible for these effects. It would be necessary to examine additional neuroanatomical sites before determining (a) brain area(s) responsible for the lordosis decline that occurs in response to the process of infusion and (b) regional specificity of the effects of the 5-HT_{1B} receptor compounds.

Although EP rats showed no decline in lordosis behavior after the process of intracranial infusion, both EO and EP rats exhibited an increase in resistance to the male's attempts to mount after infusion; but this was independent of the type of infusion and, therefore, is attributed to the infusion process rather than any effect of the 5-HT_{1B} receptor agonist. Proceptivity was also reduced by the drug; but there was little evidence of dose responsivity so this may have reflected the increased resistance rather than any specific effect on proceptive/solicitous behaviors.

Although a variety of stressors have been reported to influence female rat sexual behavior, the direction of change is not always consistent (Cecconello et al., 2009; Donadio et al., 2007; Gorzalka et al., 1998; Hulse and Coleman, 1983; Uphouse et al., 2008; Uphouse et al., 2005; White and Uphouse, 2004). It is possible that some of this inconsistency may arise from behavioral assessment at different phases of an inhibition/recovery process that is initiated by the stressor. Alternatively, the type of hormonal priming and/or strain of rat used may influence the response to stress. In ovariectomized Fischer females, either a relatively high dose of EB (50 μg/rat) or the combination of 10 μg EB plus progesterone (at least 25 μg/rat) attenuated the effect of a 5 min restraint experience (White and Uphouse, 2004). Consistent with this protective effect of female gonadal hormones, 5 to 60 min of restraint had no effect on lordosis behavior of intact, proestrous Fischer females (Uphouse et al., 2003).

It is currently unknown if these protective effects of progesterone on a mild stress-induced decline in lordosis behavior extend to rat strains other than the Fischer rat. However, anxiolytic effects of progesterone have been reported for a variety of rat strains (Frye, 2007; Llaneza and Frye, 2009; Picazo and Fernandez-Guasti, 1995; Saavedra et al., 2006; Schneider and Popik, 2007). While effective anxiolytic doses have varied with the behavioral paradigm investigated, concentrations of progesterone comparable to, or less than, those used in the current study are anxiolytic (Saavedra et al., 2006; Schneider and Popik, 2007), and intact female rats show less "anxious" behavior during stages of the cycle when both estrogen and progesterone are relatively high (Frye et al., 2000; Llaneza and Frye, 2009; Marcondes et al., 2001). Therefore, it is not unreasonable to attribute progesterone's anxiolytic actions as a contributor to its ability to reduce the lordosis-inhibitory effects of mild infusion stress.

The mechanisms responsible for progesterone's protection against effects of mild stress are not known. Although, in the VMN, progesterone acts through classical intracellular progesterone receptors to facilitate lordosis behavior (Mani et al., 1994; Ogawa et al., 1994), nonclassical genomic and ligand-independent mechanisms contribute to the effects of progesterone (Mani, 2006). Because of their ability to enhance $GABA_A$ receptors, progesterone metabolites, such as 5 alpha-pregnan-3 alpha-ol-20-one (allopregnanolone), may allow continued sexual behavior in the presence of the mild stressor. It will be interesting to determine if blockers of progesterone metabolism alter this protective effect of progesterone.

In summary, the process of intracranial infusion transiently reduced lordosis behavior of ovariectomized rats hormonally primed with estradiol benzoate but not in rats primed with estradiol benzoate and progesterone. The process, rather than the nature of the infusion, appeared to be responsible for the reduction in lordosis behavior since lordosis inhibition was independent of cannulae location and infusion content. Unexpectedly, infusion of the 5-HT_{1B} receptor agonist, CP 93129, into the VMN was unable to reverse this lordosis inibition. In fact, at the highest dose examined, VMN infusion with CP 93129 accentuated and prolonged the inhibition in EO rats. At lower doses of the drug, lordosis inhibition of EO rats was prolonged but was not enhanced relative to that of the saline infusion. Additional studies will be required to determine if these effects of CP 93129 are mediated by agonist action at 5-HT terminal autoreceptors or by actions at non 5-HT neurons within the VMN and if sites outside the VMN produce comparable effects.

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