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Effects of Sympathetic Inhibition on Receptive, Proceptive, and Rejection Behaviors in the Female Rat

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MESTON, C. M., I. V. MOE AND B. B. GORZALKA. *Effects of sympathetic inhibition on receptive, proceptive, and rejection behaviors in the female rat.* PHYSIOL BEHAV **59**(3) 537–542, 1996.—The present investigation was designed to examine the effects of sympathetic nervous system (SNS) inhibition on sexual behavior in ovariectomized, steroid-treated female rats. Clonidine, an alpha$_2$-adrenergic agonist, guanethidine, a postganglionic noradrenergic blocker, and naphazoline, an alpha$_2$-adrenoreceptor agonist were used to inhibit SNS activity. Intraperitoneal injections of either 33 μg/ml or 66 μg/ml clonidine significantly decreased receptive (lordosis) and proceptive (ear wiggles) behaviors and significantly increased rejection behaviors (vocalization, kicking, boxing). Either 25 mg/ml or 50 mg/ml guanethidine significantly decreased receptive and proceptive behavior and had no significant effect on rejection behaviors. Naphazoline significantly inhibited lordosis behavior at either 5 mg/ml or 10 mg/ml doses, significantly inhibited proceptive behavior at 5 mg/ml, and had no significant effect on rejection behaviors. These findings support the hypothesis that SNS inhibition decreases sexual activity in the female rat.

Lordosis Proceptive behavior Rejection behavior Clonidine Guanethidine Naphazoline Alpha-adrenergic

INTRODUCTION

IT IS becoming increasingly apparent that central nervous system regulation of norepinephrine (NE) may exert a prominent influence on lordosis behavior in the female rat. Research indicates that hormonal activation of female reproductive behavior is accompanied by increased hypothalamic NE release (22), and lesions which destroy NE input to the hypothalamus also inhibit the ability of estrogen and progesterone to facilitate lordosis responding (9,10). One primary regulator of NE release is the alpha$_2$-adrenoreceptor. Numerous studies have demonstrated that alpha$_2$-adrenoreceptor activation can substantially decrease NE release from various central and peripheral sites (12). Reports have also revealed that drugs which act as alpha$_2$-adrenoreceptor agonists diminish lordosis behavior and those which act as alpha$_2$-adrenoreceptor antagonists facilitate lordosis responding. Specifically, clonidine, a selective alpha$_2$-adrenergic agonist, has been reported to suppress lordosis responding when administered both centrally, at 0.5 μg and 1.0 μg doses (1), and peripherally at doses ranging from 20 μg/kg to 100 μg/kg (2,18). Central administration of norepinephrine and epinephrine at doses as low as 2 μg/animal also inhibits lordosis behavior (1). Administration of the alpha$_2$-adrenergic antagonist yohimbine, by contrast, has been shown to facilitate receptive behavior (7) and to block the reduction in lordosis levels after injections of both clonidine (2) and NE (1). Etgen and colleagues (5,6) have described in detail the mechanism of action by which NE, in concert with oxytocin, acts to increase the overall excitability of hypothalamic neurons and subsequently to influence reproductive behavior in the female rat.

While the effects of alpha-adrenergic activity on lordosis responding via central mechanisms have been well documented, the possible contributory role of adrenergic mechanisms on sexual responding via peripheral mechanisms has been almost entirely ignored. It is well known that activation of presynaptic alpha$_2$-adrenergic receptors, via drugs such as clonidine, inhibits the release of NE from postganglionic sympathetic nerve endings and blockade of alpha$_2$-adrenergic receptors, via antagonists such as yohimbine, potentiate the release of NE from nerve endings and, thereby, increase sympathetic outflow (11,21). Moreover, following microiontophoretic application, clonidine, and to a

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lesser degree epinephrine and NE, block the spontaneous firing of preganglionic sympathetic neurons (8). Since preganglionic neurons are cholinergic, this indicates that alpha$_2$-adrenoreceptors in preganglionic neurons cannot be considered autoreceptors. Therefore, in addition to acting as an agonist at postganglionic autoreceptors, clonidine may inhibit sympathetic activity by acting as a preganglionic alpha$_2$-adrenergic antagonist. Furthermore, alpha$_2$-adrenoreceptors are reportedly involved in increasing vagal tone, facilitating platelet aggregation, regulating metabolic effects, mediating the contraction of some arteries and veins, and promoting vasodilation in certain vascular beds by stimulating the release of endothelium-derived relaxing factor. Although the lordosis-inhibiting effects of clonidine in the literature have been generally interpreted in terms of central adrenergic mechanisms, it is possible that clonidine influences sexual activity by acting, at least in part, through direct sympathetic inhibition. Recent research in humans has shown that activation of the sympathetic nervous system (SNS) facilitates sexual arousal in women, including increases in both vaginal blood volume and vaginal pulse amplitude (16,17). The present investigation was undertaken to examine the effects of SNS inhibition on sexual responding in the female rat.

Experiment 1 was designed to extend the findings of previous research on the effects of clonidine on sexual responding by examining the influence of moderate and high doses of peripherally administered clonidine on three independent measures of sexual behavior in the female rat: sexual receptivity (lordosis quotient), proceptivity (ear wiggling), and rejection behavior (vocalization, kicking, boxing). By exploring the effects of clonidine on active sexual rejection behaviors, this study examined the possibility that previously reported decreases in lordotic responding following clonidine administration (1,2,18) may have been secondary to general sedative effects of clonidine (3,4,14), rather than changes in sexual interest per se. A concurrent decrease in receptive and proceptive behaviors and increase in rejection behaviors would suggest that clonidine is not acting to inhibit sexual behavior through sedative influences. Previous reports of clonidine effects on sexual activity in the female rat (1,2,18) were restricted to an examination of lordosis behavior.

In Experiment 2, the effects of moderate and high doses of guanethidine on receptive, proceptive, and rejection behaviors were examined. Guanethidine is a sympathetic postganglionic blocker (13) which acts by displacing norepinephrine from storage vesicles in the peripheral adrenergic neuron (19). This action appears to be selective for NE storage vesicles of the SNS (19). Thus, unlike clonidine which exerts an influence at both central and peripheral levels, guanethidine allows for the selective examination of peripheral SNS inhibition on sexual responding. Experiment 3 examined the effects of moderate and high doses of naphazoline on receptive, proceptive, and rejection behaviors in the female rat. Naphazoline is an alpha$_2$-adrenoreceptor agonist which, like guanethidine, has minimal ability to cross the blood–brain barrier (15). If guanethidine and naphazoline suppress sexual responding, this would be consistent with a moderating role of peripheral SNS inhibition on sexual activity. Moreover, because naphazoline, like clonidine, acts selectively as an alpha$_2$-adrenoreceptor agonist but, unlike clonidine, does not act centrally, an inhibitory influence of naphazoline on sexual responding would suggest that previous reports of an inhibition of lordosis by clonidine may, in part, be explained by a suppression of peripheral SNS activity. On the other hand, if naphazoline increases or fails to influence sexual responding, the findings will provide support for a primarily centrally acting inhibitory influence of clonidine on sexual behavior.

METHOD

Animals

Fifteen, 2-month-old Long–Evans female rats (Charles River Inc., Quebec, Canada) were used to conduct Experiment 1, and 14 6-mo-old Long Evans female rats (Charles River) were used to conduct Experiments 2 and 3. All female animals were bilaterally ovariectomized using sodium pentobarbital (44 μg/kg) and ketamine (40 μg/kg) anesthesia 2 wk prior to testing. The rats were experimentally but not sexually naive. Six stimulus male rats, aged 5 mo on the first day of testing, were used to elicit sexual responding. All animals were maintained in a temperature controlled room (21 ± 1°C) and kept on a 12:12 reverse light/dark cycle (lights on at 2100 h). The male animals were housed in wire mesh cages in groups of 3–4, female animals were housed in groups of 5. Food (Richmond Standard diet, 5012) and water were available ad lib. Tests of sexual behavior were conducted in an illuminated room approximately 6 h after commencement of the dark period.

Drug Injection and Behavioral Testing

All female animals received three tests of sexual behavior (1 test per wk). Ovariectomized females were injected subcutaneously with estradiol benzoate (10 μg/0.1 ml of peanut oil) 48 h prior to testing, followed by progesterone (200 μg/0.1 ml peanut oil) 4 h prior to testing. In Experiment 1, one half h before testing, test females were injected intraperitoneally with either saline (1 ml/kg body weight) or one of two doses of clonidine hydrochloride (Sigma Chemical Co., St. Louis, MO). Clonidine (33 μg/ml or 66 μg/ml) was dissolved in physiological saline and injected in a volume of 1 ml/kg body weight. Test females in Experiment 2 were injected intraperitoneally with either saline (1 ml/kg body weight) or one of two doses of guanethidine (Sigma), one half h prior to testing. Guanethidine, dissolved in physiological saline (25 mg/ml or 50 mg/ml), was injected in a volume of 1 ml/kg body weight. In Experiment 3, one half h prior to testing, test females were injected intraperitoneally with either saline or one of two doses of naphazoline (Sigma) dissolved in physiological saline (5 mg/ml or 10 mg/ml). All treatments were injected in a volume of 1 ml/kg body weight. The experimenter was blind to which treatments the animals received.

Individual subjects were placed with a sexually vigorous male rat in a 43 cm (height) $\times$ 27 cm (diameter) glass testing arena. Prior to testing, male rats were stimulated by placing highly receptive (stimulus) females above their transport cage for 10 min. Males were then placed in the testing chambers for a five minute habituation period and allowed to mount with one stimulus female prior to testing.

The lordosis response of each female was scored quantitatively by recording the presence or absence of lordosis in response to a mount with pelvic thrusting by the male. Following the occurrence of 10 mounts with pelvic thrusting by the male, females were removed from the testing arenas and lordosis quotients were calculated (no. of lordosis responses/no. of mounts $\times$ 100). In addition to lordosis behavior, female proceptive and rejection behaviors were also recorded. Proceptivity was measured by counting the number of ear wiggles. A rejection score of 0–3 was assigned to each female. A score of 0 was given to rats who showed no rejection, a score of 1 was given to rats who displayed vocal rejection, a score of 2 was given to rats who rejected vocally and kicked, and a score of 3 was given to animals with maximum rejection including vocal rejection, kicking, running away, jumping, and fighting.

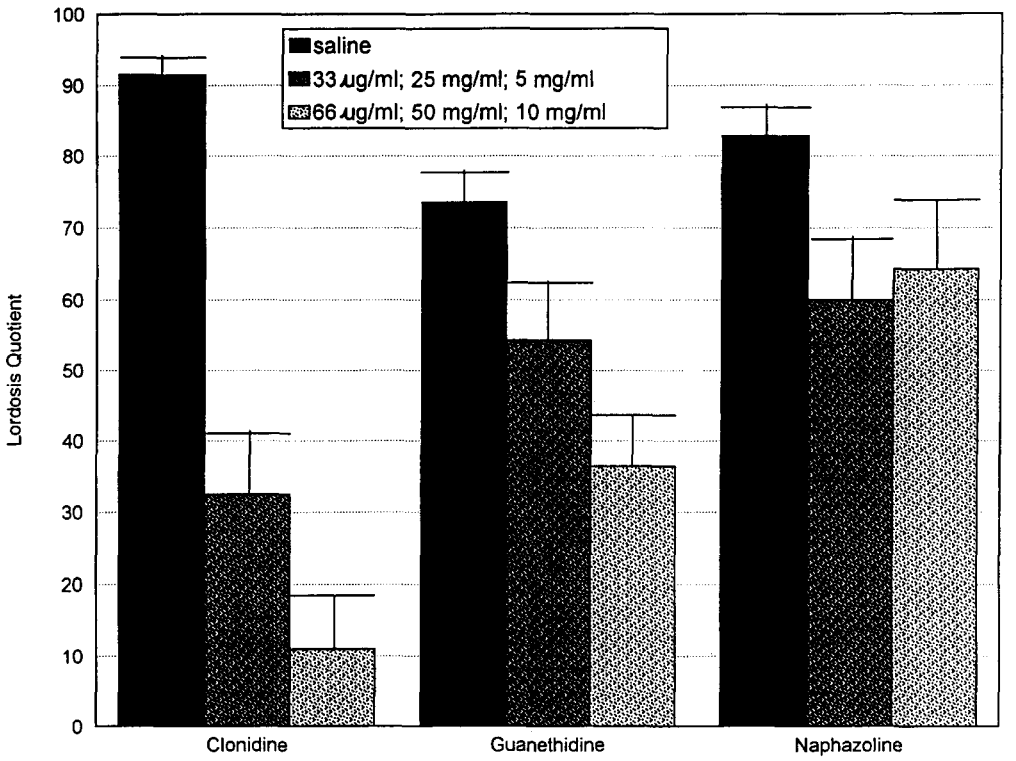

FIG. 1. Mean (±SEM) lordosis quotients (no. of lordosis responses/no. of mounts with pelvic thrusting × 100) in ovariectomized rats following saline, 33 µg/ml and 66 µg/ml clonidine, 25 mg/ml and 50 mg/ml guanethidine, and 5 mg/ml and 10 mg/ml naphazoline, treatments. The three tests were separated by 1 wk and were preceded by injections of 10 µg/0.1 ml estradiol benzoate 48 h prior to testing, and 500 µg/0.1 ml progesterone 4 h prior to testing.

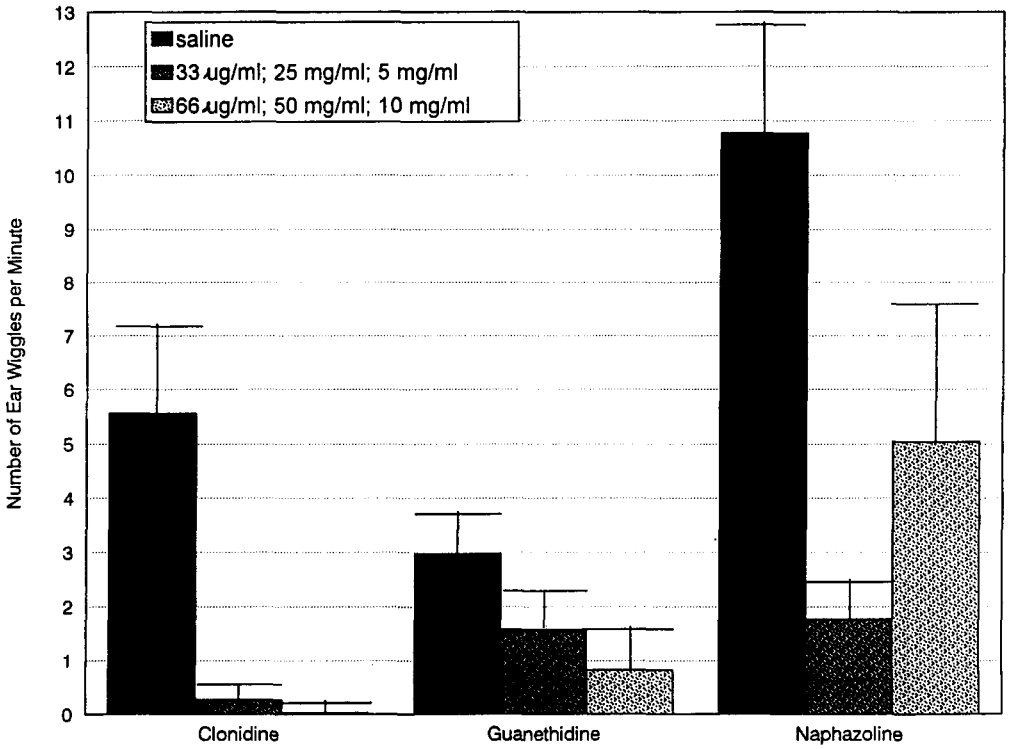

FIG. 2. Mean (±SEM) number of ear wiggles per minute in ovariectomized rats following saline, 33 µg/ml and 66 µg/ml clonidine, 25 mg/ml and 50 mg/ml guanethidine, and 5 mg/ml and 10 mg/ml naphazoline, treatments. The three tests were separated by 1 wk and were preceded by injections of 10 µg/0.1 ml estradiol benzoate 48 h prior to testing, and 500 µg/0.1 ml progesterone 4 h prior to testing.

Data Analysis

Lordosis and proceptive behaviors were analyzed using repeated measures analysis of variance. Subsequent pairwise comparisons were analyzed using Tukey's HSD test for differences among group means. Rejection behaviors were analyzed using Friedman analysis of variance with follow-up pairwise comparisons.

RESULTS

Experiment 1: Clonidine

A one-way repeated measures analysis of variance revealed clonidine had a significant inhibitory effect on lordosis behavior, $F(2, 18) = 20.41$, $p < 0.0001$. Pairwise comparisons indicated significant decreases in lordotic responding with both 33 μg/ml of clonidine, $p < 0.001$, and 66 μg/ml clonidine, $p < 0.001$. There was no significant difference in lordosis behavior between the 33 μg/ml clonidine and 66 μg/ml clonidine treatment groups. Mean lordotic responses ($\pm$SEM) are presented in Fig. 1.

Results from a one-way repeated measures analysis of variance conducted on proceptive behavior indicated a significant main effect of clonidine, $F(2, 18) = 8.83$, $p < 0.01$. Follow-up analysis using Tukey's HSD test revealed significant decreases in ear wiggling behavior, in comparison to the saline control group, with both 33 μg/ml clonidine, $p < 0.01$, and 66 μg/ml clonidine, $p < 0.01$. Proceptive responses did not differ significantly between the 33 μg/ml clonidine and 66 μg/ml clonidine treatment groups. Mean ear wiggling responses ($\pm$SEM) are presented in Fig. 2.

Rejection behaviors were significantly increased with clonidine $F_r(2) = 10.85$, $p = .004$. Follow-up analyses revealed a significant difference in rejection behaviors between the saline and 33 μg/ml, and between the saline and 66 μg/ml, clonidine treatment groups. There was no significant difference in rejection behavior between the 33 μg/ml clonidine and 66 μg/ml clonidine treatment groups. Mean rejection responses ($\pm$SEM) are presented in Fig. 3.

Experiment 2: Guanethidine

Results from a one-way repeated measures analysis of variance revealed guanethidine had a significant inhibitory effect on lordosis behavior, $F(2, 26) = 9.76$, $p < 0.001$. Pairwise comparisons indicated guanethidine significantly decreased lordotic responding at a dose of 50 mg/ml, and showed a nonsignificant trend toward decreasing lordotic responding at 25 mg/ml, $p = 0.07$. Lordosis behavior did not differ significantly between the 25 mg/ml and 50 mg/ml guanethidine treatment groups. Mean lordotic responses ($\pm$SEM) are presented in Fig. 1.

An analysis of variance conducted on proceptive behavior indicated a significant main effect of guanethidine, $F(2, 26) = 8.65$, $p = 0.003$. Follow-up analysis revealed significant decreases in ear wiggling behavior with both 25 mg/ml and 50 mg/ml guanethidine, $p < 0.05$. Proceptive responses did not differ significantly between the 25 mg/ml and 50 mg/ml guanethidine treatment groups. Mean ear wiggling responses ($\pm$SEM) are presented in Fig. 2. Rejection behaviors were not significantly affected with guanethidine treatment, $F_r(2) = .321$, $p = .852$. Mean rejection responses ($\pm$SEM) are presented in Fig. 3.

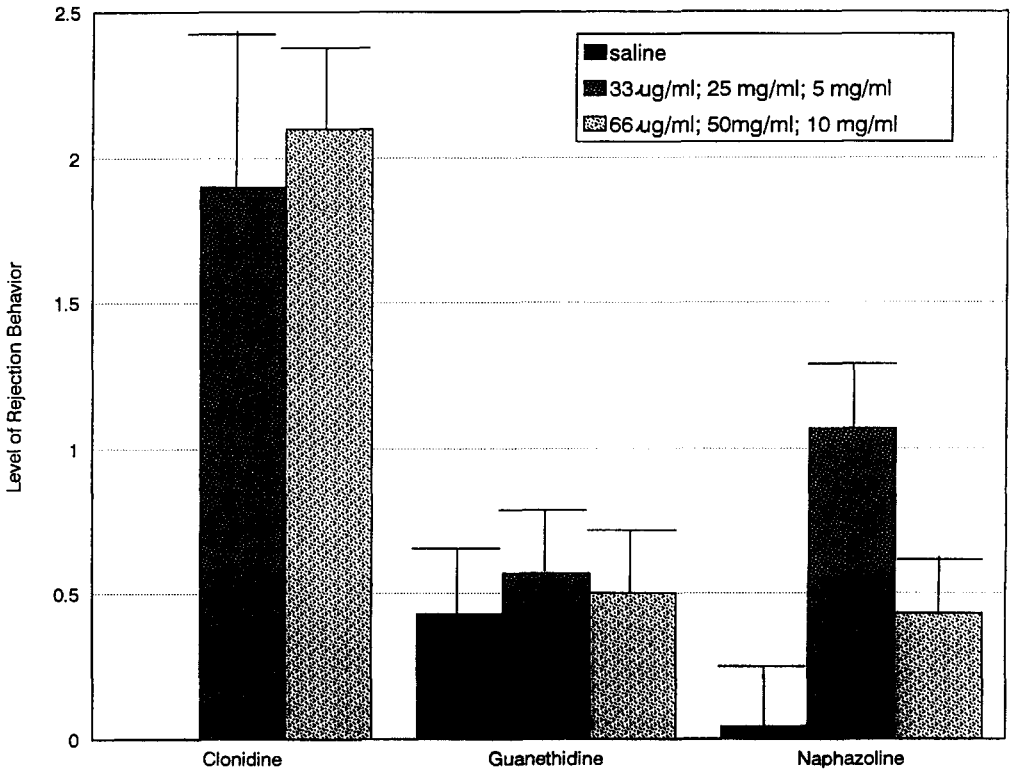

FIG. 3. Mean ($\pm$SEM) frequency of rejection behavior in ovariectomized rats following saline, 33 μg/ml and 66 μg/ml clonidine, 25 mg/ml and 50 mg/ml guanethidine, and 5 mg/ml and 10 mg/ml naphazoline, treatments. The three tests were separated by 1 wk and were preceded by injections of 10 μg/0.1 ml estradiol benzoate 48 h prior to testing, and 500 μg/0.1 ml progesterone 4 h prior to testing.

Experiment 3: Naphazoline

A one-way repeated measures analysis of variance revealed naphazoline had a significant inhibitory effect on lordosis behavior, $F(2, 26) = 6.97$, $p = 0.004$. Pairwise comparisons indicated significant decreases in lordotic responding with both 5 mg/ml of naphazoline, $p = 0.007$, and 10 mg/ml naphazoline, $p = 0.011$. There were no significant differences in lordosis behavior between the 5 mg/ml and 10 mg/ml naphazoline treatment groups. Mean lordotic responses (±SEM) are presented in Fig. 1.

Results from an analysis of variance conducted on proceptive behavior indicated a significant main effect of naphazoline, $F(2, 26) = 5.953$, $p = 0.007$. Tukey's HSD test revealed significant decreases in ear wiggling behavior, in comparison to the saline control group, with 5 mg/ml, $p = 0.006$, but not 10 mg/ml, $p = .117$, naphazoline. Proceptive responses did not differ significantly between the 5 mg/ml and 10 mg/ml naphazoline treatment groups. Mean ear wiggling responses (±SEM) are presented in Fig. 2. Naphazoline had no significant effect on rejection behaviors, $F_r(2) = .400$, $p = .135$.

DISCUSSION

Results from Experiment 1 of the present study confirm previous findings of decreased lordosis behavior following clonidine administration (1,2,18). The findings are extended by our observation that clonidine also inhibits proceptivity and increases rejection behaviors. These results were apparent for both moderate (33 μg/ml) and high (66 μg/ml) doses of clonidine. The finding that clonidine increased rejection behaviors suggests that the decrease in receptive and proceptive behaviors are not simply secondary to a more general suppression of behavior resulting from clonidine administration. That is, if the decreases in sexual behavior were solely attributable to sedative effects of clonidine (3,4,14), one would not expect to see the reported increase in active behaviors such as kicking and boxing. Moreover, there was no indication that clonidine inhibited motor activity at either dose. While it has been reported that clonidine increases aggressive behavior in mice (20), it is unlikely that this could explain the reported increase in rejection behaviors, given that rats in the present study did not show increases in aggressive behavior. When paired with same-sexed animals or when males were not attempting to mount, females failed to show any of the common indices of aggression (i.e., unprovoked biting or attacking other animals). Together, the results of the present investigation provide further support for an inhibitory role of clonidine on sexual behavior in the female rat.

In Experiment 2, guanethidine significantly inhibited receptive and proceptive behaviors at both moderate (25 mg/ml) and high (50 mg/ml) doses. In Experiment 3, naphazoline also significantly inhibited receptive behaviors at moderate (5 mg/ml) and high (10 mg/ml) doses, and significantly suppressed proceptive behaviors at moderate doses. Despite decreases in receptive and proceptive behaviors, both guanethidine and naphazoline showed only a slight but nonsignificant increase in rejection behaviors. While this does not allow us to rule out the possibility that guanethidine and naphazoline acted to inhibit sexual responding via a general nervous system suppressant effect, if these drugs were acting solely via sedative effects to influence sexual behavior, one would expect to also see a decrease in rejection behaviors.

The fact that clonidine, guanethidine, and naphazoline all suppressed lordosis and proceptive behaviors while only clonidine significantly increased rejection behaviors, leads one to question whether these behaviors may be differentially influenced by central and peripheral mechanisms. It is possible that rejection behaviors are largely centrally mediated, hence their alteration by clonidine, a drug which exerts significant central as well as peripheral adrenergic activity, but not by guanethidine and naphazoline which primarily have a peripheral mechanism of action. Receptive and proceptive behaviors, on the other hand, may be mediated primarily by peripheral adrenergic events, or by both peripheral and central adrenergic events, hence their alteration by both peripherally acting (guanethidine and naphazoline) and centrally and peripherally acting (clonidine) drugs.

Because guanethidine and naphazoline act to selectively inhibit peripheral sympathetic outflow without influencing adrenergic mechanisms at a central level, the results of this study suggest that inhibition of the SNS may inhibit sexual behavior in the female rat. This would suggest that increased SNS activity may facilitate sexual activity in the rat. Consistent with this hypothesis is a report that peripheral administration of epinephrine increases lordosis behavior (23). In addition, given naphazoline's selective influence on alpha$_2$-adrenoreceptors, the findings of Experiment 3 suggest that the inhibitory influence of clonidine on lordosis behavior, noted in both the present and past (1,2,18) investigations, may be partially attributable to peripheral adrenergic-mediated events. Future studies which examine the effects of clonidine on sexual responding following selective destruction of central alpha$_2$-adrenergic nerve terminals would provide further insight into the peripheral as opposed to central influences of clonidine and other alpha-adrenergic agonists on sexual responding.

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