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THE ROLE OF NOREPINEPHRINE IN THE EXPRESSION OF LORDOSIS IN FEMALE RATS

presented by

BECKY L. DAVIS

has been accepted towards fulfillment of the requirements for

M.S. degree in ZOOLOGY

Major professor

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# THE ROLE OF NOREPINEPHRINE IN THE EXPRESSION OF LORDOSIS IN FEMALE RATS

By

Becky L. Davis

A THESIS

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ABSTRACT

THE ROLE OF NOREPINEPHRINE IN THE EXPRESSION OF LORDOSIS IN FEMALE RATS

By

Becky L. Davis

The importance of NE for lordosis in female rats and its site of action in hormone-sensitive brain regions controlling receptivity were examined. Ovariectomized, hormone-treated female rats underwent surgical procedures and/or drug treatments and were tested for sexual receptivity. It was found that systemic injection of either an $\alpha_1$- or ß-antagonist failed to block lordosis in receptive females and injection of NE into the VMN of unreceptive females failed to facilitate lordosis. Neurotoxic lesions of the ventral noradrenergic bundle (VNAB) reduced NE concentrations in the VMN and MPN and inhibited lordosis. Lordosis was restored with i.c.v. administration of an $\alpha_1$-receptor agonist. Neurotoxin injection into either the VMN or MPN reduced NE concentrations in these nuclei, but failed to alter lordosis. Furthermore, injection of the $\alpha_1$ agonist into either the VMN or MPN of VNAB-lesioned females failed to reinstate lordosis. These results indicate that while NE is important for lordosis, noradrenergic neurons terminating in either the VMN or MPN are not essential for hormonal induction of sexual receptivity in female rats.

*To my loving and thoughtful mother, Carolyn J. Burke*

**ACKNOWLEDGEMENTS**

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## INTRODUCTION

It has been well established that the interaction of estrogen and progesterone are required for the full expression of sexual behaviors in female rats (Beach, 1942; Young, 1961; Edwards, et al., 1968; Whalen, 1974). Proceptive behaviors such as ear-wiggling and hopping/darting movements are used by the female rat to attract the male (Beach, 1976). Receptivity of the female is evaluated by observing the lordosis reflex (Beach, 1976). This is an arched posture the receptive female adopts when mounted by the male and maximizes the probability that successful intromission will be achieved. Proceptive behaviors and lordosis are thought to be controlled by separate mechanisms. Whalen has demonstrated that the probability of lordosis behavior occurring in ovariectomized female rats is positively correlated with increases in both estrogen and progesterone (Whalen, 1974). However, proceptive behaviors are not greatly effected by increases in estrogen but are strongly correlated with progesterone dose (Whalen, 1974). These studies suggest that lordosis and ear-wiggling and hopping/darting behaviors are separately effected by estrogen and progesterone. Differences in control could occur

in separate brain regions due to differences in the action of estrogen and progesterone on various neurotransmitter systems.

To examine the inhibitory effects of a treatment on proceptivity or lordosis, ovariectomized female rats are treated with high doses of estrogen followed by progesterone. This hormone treatment is thought to mimic the natural state of estrous during which the female is receptive. If a given treatment acts to block mechanisms of receptivity, the female will not display some aspects of sexual behavior. Conversely, in order to examine facilitation of a treatment on sexual behavior, ovariectomized females are treated with low doses of estrogen with or without subsequent progesterone administration. This protocol exposes the animal to sub-threshold levels of hormones and females do not exhibit proceptive behaviors or lordosis when exposed to a male. Through the use of these models, compounds can be evaluated for their role in mediating sexual behavior. By determining the location and functional mechanisms of steroid responsive neural circuits which control reproductive behaviors researchers are able to gain incite into the action of steroids on brain function.

Much work has been done on the brain circuitry involved in sexual behaviors in the female rat. The location of steroid-concentrating neurons in the central nervous system suggests a pattern of circuitry functioning to control all aspects of reproduction (Stumpf, et al., 1975; Pfaff and

Keiner, 1973). Through olfactory and visual cues, the estrous female senses the males presence and will begin proceptive behaviors to increase the males interest. As the aroused male begins tactile contact and eventual mounting, the information attained by the female through somatosensory stimulation is transmitted via ascending pathways up the spinal cord to the midbrain (for review see Pfaff, 1980). It is thought that estrous-relevant information from the hypothalamus is coordinated with motor output at the level of the midbrain, resulting in lordosis.

Estrogen-concentrating neurons are found throughout the spinal cord, medulla, and midbrain. These neurons could be involved in relaying both ascending somatosensory input as well as part of the descending motor output. High numbers of estrogen-concentrating neurons are also located in the hypothalamus, preoptic area, and limbic regions, areas known to be involved in many of the physiologic as well as behavioral aspects of reproduction. The distribution of progestin receptors in the female rat brain also appears to be widespread but high concentrations of estrogen-inducible progestin receptors are found only in the preoptic region and medial basal hypothalamus (primarily in the lateral part of the ventromedial nucleus), and to a lesser extent in limbic structures (Maclusky and McEwen, 1980; Parsons, et al., 1982). A small percentage of the progestin receptor-containing neurons in the preoptic and medial basal hypothalamus also

project to the midbrain (DonCarlos and Morrell, 1990). All of these areas of the brain could be required to coordinate the complex physical and behavioral requirements for successful reproduction. These estrogen- and progestin-concentrating regions correspond to sites in the brain where hormone implants and lesions effect the sexual receptivity of ovariectomized female rats.

Manipulations within the ventromedial nucleus (VMN) of the hypothalamus appear to have dramatic effects on the sexual behavior of ovariectomized female rats. Concentrated estrogen implants into the VMN will induce lordosis without progesterone treatment (Barfield, et al., 1983), while cholesterol-diluted estrogen (a concentration more likely to mimic physiological conditions) will produce a priming effect and rats become receptive following systemic progesterone injection (Rubin and Barfield, 1980; Davis, et al., 1982). In support of the theory that estrogen acts in the VMN to facilitate lordosis it was demonstrated that implants of an antiestrogen into the VMN of female rats reduces lordosis in females injected with estrogen and progesterone (Meisel, et al., 1987). Progesterone also appears to have a major effect on receptivity when implanted into the VMN. Female rats primed with low doses of estrogen exhibit lordosis following implants of progesterone into the VMN (Rubin and Barfield, 1983; Pleim and Barfield, 1988). Furthermore, antiprogestins reduced progesterone-induced lordosis when implanted into the

VMN (Etgen and Barfield, 1986). In addition, electrolytic destruction of the lateral aspect of the VMN abolished estrogen- and estrogen/progesterone-induced lordosis (Mathews and Edwards, 1977; Pfaff and Sakuma, 1979; Mathews, et al., 1983), although some females did regain the ability to display lordosis behavior (LaVaque and Rodgers, 1975; Okada, et al., 1980). On the other hand, very large lesions that encompass the entire VMN failed to interrupt hormone-induced receptivity (Okada, et al., 1980). Evidence suggests that the VMN may be a major site for the action of estrogen and progesterone to mediate sexual behavior, but the contribution of other sites in the circuit must also be considered.

Another brain region containing a high concentration of steroid hormone receptors is the preoptic area (POA). Some investigators have found that implantation of estrogen in the POA is effective in inducing receptivity, although spread of the concentrated estrogen to the VMN can not be ruled out (Lisk, 1962; Yanase and Gorski, 1976). In contrast however, Barfield and coworkers failed to observe an effect of dilute estrogen implants in the POA of ovariectomized female rats (Barfield, et al., 1983). Furthermore, implants of antiestrogen into the POA failed to block estrogen/progesterone-induced receptivity as it had in the VMN (Meisel, et al., 1987). Also, unlike the VMN, progesterone implants into the POA failed to have an effect on the receptivity of female rats primed with low dose estrogen

(Rubin and Barfield, 1983; Pleim and Barfield, 1988) and antiprogestins implanted into the POA failed to reduce progesterone-induced lordosis (Etgen and Barfield, 1986).

As with the implant studies, lesions of the POA produced different results than did VMN lesions. Powers and Valenstein have shown that in estrogen/progesterone-treated ovariectomized female rats, less estrogen is required to induce lordosis in females with lesions in the medial preoptic area (MPOA) (Powers and Valenstein, 1971). Bast demonstrated that lesions in the medial preoptic nucleus (MPN) reduced the receptivity induced by estrogen implants into the VMN (Bast, et al., 1987). In addition, Leedy showed that the effect of MPOA lesions on lordosis is dependant on location. Small lesions in the ventral portion of the MPN were found to facilitate receptivity in female rats, whereas lesions placed more dorsally inhibited lordosis in ovariectomized estrogen/progesterone-treated females (Leedy, 1984). These results suggest that while neurons in the VMN are important in the facilitation of lordosis, neurons of the MPOA may be facilitatory and inhibitory with regard to their role in mediating hormone effects on receptive behaviors.

Other regions of the brain which also concentrate steroid hormones may also be important for the control of lordosis. Lesions in estrogen-concentrating regions such as the amygdala (Masco and Carrer, 1980), zona incerta (Dornan, et al., 1991), and midbrain areas (Herndon, 1976; Edwards and Pfeifle, 1981;

Yamanouchi and Arai, 1982 and 1985) have been shown to affect lordosis. Lesions in some areas of the midbrain have produced dramatic deficits in lordosis behavior. It may be that pathways mediating sensory input or information coordinating motor output important for the lordosis reflex are being disrupted by lesions in these brain regions. Conversely, implants of dilute estrogen into estrogen-concentrating brain regions such as the diagonal band of Broca, lateral habenula, amygdala, cortex (Barfield, et al., 1983), mesencephalic reticular formation, and caudate-putamen (Yanase and Gorski, 1976) were ineffective in inducing receptivity. Others have, however, facilitated lordosis in estrogen primed female rats with progesterone implants in midbrain regions such as the ventral tegmental area (VTA) (Luttge and Hughes, 1976; Tennent, et al., 1982) and mesencephalic reticular formation (Ross, et al., 1971; Yanase and Gorski, 1976), as well as the habenula (Tennent, et al., 1982), hippocampus, and amygdala (Franck and Ward, 1981). In contrast, other investigators have failed to see an effect of progesterone implants into the midbrain on lordosis (Rubin and Barfield, 1983; Pleim and Barfield, 1988). Pleim, et al., have recently shown that contralateral implants of progesterone in the VMN and VTA are more effective at inducing lordosis in estrogen-primed females than a unilateral implant in the VMN alone (Pleim, et al., 1991). Although studies evaluating the effects of steroid implants and lesions in regions of steroid-concentrating

neurons are somewhat ambiguous and inconsistent, these studies do provide evidence that neural systems which control lordosis are located throughout the brain.

Steroid hormones act in many ways to effect neural communication. One way in which estrogen and progesterone produce their changes on neurons in the lordosis circuit is through action on specific neurotransmitter systems. Most of the known neurotransmitters and neuromodulatory peptide systems have been examined for their effects on lordosis. Some, such as the cholinergic neurons, appear to be directly involved in the ability to lordose since cholinergic antagonists can dramatically block receptivity in estrogen/progesterone treated female rats (Clemens, et al., 1980; Dohanich and Clemens, 1981). The involvement of neuropeptide (ie. opiates, LHRH, substance P) and monoamine (ie. NE, DA, 5HT) neurotransmitters in the lordosis circuit are much less clear (for an overview see: Meyerson, et.al. 1985; De Vries, 1990).

One of the first neurotransmitters to be examined for its role in receptivity was norepinephrine. Several lines of evidence suggest that hypothalamic noradrenergic neurons mediate the stimulatory effects of gonadal steroids on sexual receptivity in female rats. Some investigators have shown that microinjection of norepinephrine or adrenergic receptor agonists directly into the VMN (Foreman and Moss, 1978; Fernández-Guasti, et al., 1985) or MPN (Foreman and Moss,

1978) induce lordosis in ovariectomized, estrogen-treated female rats. Neurotoxin-induced lesions of hypothalamic noradrenergic neurons impair lordosis in ovariectomized, estrogen/progesterone-treated female rats (Hansen, et al., 1981). In addition, NE release in the VMN is elevated in ovariectomized, estrogen/progesterone-treated rats displaying high levels of lordosis behavior (Vathy and Etgen, 1989).

Noradrenergic innervation of the hypothalamus originates from perikara located in subcoeruleus nuclei of the pons-medulla (i.e. A1, $A_2$, $A_5$, $A_7$; Dählström and Fuxe, 1964; Lindvall and Björklund, 1983). Axons of these neurons ascend to the diencephalon via the ventral noradrenergic bundle (VNAB) and terminate in virtually all regions of the hypothalamus including the VMN and MPN (Olsen and Fuxe, 1972; Jacobowitz and Palkovits, 1974; Moore and Bloom, 1979; Palkovits, 1981). These noradrenergic neurons also concentrate estrogen and project to regions containing a high number of estrogen-concentrating neurons (Heritage, et al., 1977).

Ovarian hormones have been shown to affect noradrenergic transmission both pre- and post-synaptically. Evidence suggests that estrogen and progesterone may influence the uptake and release of norepinephrine from synaptosomes (Janowsky and Davis, 1970). In addition, estrogen and progesterone influence the levels of KCl-stimulated NE release in the VMN of anesthetized female rats (Vathy and Etgen,

1988). Priming doses of estrogen have also been shown to influence norepinephrine turnover in related areas such as the diagonal band of Broca, periventricular nucleus and lateral septum (Renner, et al., 1986). Further studies show that NE levels are elevated in the VMN of receptive females when exposed to a male (Vathy and Etgen, 1989). Lastly, estrogen has been shown to increase the firing rate of $A_1$ noradrenergic neurons which project to the hypothalamus (Kaba, et al., 1983). These findings suggest that steroid hormones may influence NE transmission by their direct presynaptic action on the noradrenergic neuron.

In addition to the direct actions on noradrenergic neurons, ovarian hormones can also affect noradrenergic transmission by modulating postsynaptic adrenergic receptors on hormone-concentrating target neurons. Estrogen and progesterone have been shown to differentially alter the binding characteristics of selective radioligands to $\alpha_1$-, $\alpha_2$-, and ß-adrenergic receptors in the amygdala and hypothalamus of ovariectomized female rats (Agnati, et al., 1980). Estrogen effects the density of $\alpha_1$-adrenergic receptors in the MPN, median eminence and VMN and alters the diurnal rhythm of $\alpha_1$-receptor density in the MPN and median eminence (Weiland and Wise, 1987; Sortino, et al., 1989). These studies provide evidence that estrogen and progesterone, at least in part, act at the level of the adrenergic receptor to produce changes in

noradrenergic transmission which modulates behavioral responses.

It is conceivable that the noradrenergic system could be one of the components of the lordosis circuit and might function to transmit incoming information from the spinal cord and brain stem to other brain regions such as the hypothalamus. The responsiveness of this pathway could be acted upon by estrogen and/or progesterone to coordinate information important for the performance of receptive behavior. The overall purpose of the following experiments was to evaluate the importance of NE for hormone dependant receptivity and to determine the location of NE influence within the brain circuity controlling reproductive behaviors.

**EXPERIMENT 1: SYSTEMIC INJECTION OF EITHER $\alpha_1$- OR ß-ADRENERGIC ANTAGONISTS FAIL TO INHIBIT LORDOSIS IN ESTROGEN/PROGESTERONE TREATED FEMALE RATS**

Although NE is believed to be important for the control of reproductive behaviors in the female rat, the specific adrenergic receptor subtype(s) involved and the role of these receptors in mediating lordosis is unclear. Through the use of pharmacologic manipulations, noradrenergic receptors have been traditionally classified into $\alpha_1$-, $\alpha_2$- and ß-adrenergic receptors. It is generally accepted that $\alpha_1$- and ß-receptors are located on the postsynaptic neuron and produce their effects through second messenger systems utilizing phosphoinositide hydrolysis and adenylate cyclase, respectively. Alpha-2 receptors are presumably located on the presynaptic terminal of the noradrenergic neuron and function to inhibit the release of NE. However, there are some $\alpha_2$ type receptors located postsynaptically in the brain as well (Janowsky and Sulser, 1987).

To establish the importance of either $\alpha_1$- or ß-adrenergic receptors for the transmission of information resulting in sexual behavior, the pharmacological agents phenoxybenzamine or propranolol were used to block these receptors in estrogen/progesterone-treated female rats. Phenoxybenzamine is an $\alpha$-receptor antagonist and is moderately selective for the $\alpha_1$-adrenergic receptor. Propranolol is a nonselective, ß-adrenergic receptor antagonist (Weiner, 1985). Both compounds penetrate into the brain and produce their effects by binding

to their specific receptors thereby blocking the binding and action of endogenous norepinephrine.

The purpose of the present study was to determine if estrogen/progesterone-induced lordosis is mediated by the stimulation of either the $\alpha_1$- or ß-adrenergic receptor, since steroids have been shown to affect the binding characteristics of these receptor types (Agnati, et al., 1980). This study demonstrates that systemic injections of either the $\alpha_1$-receptor antagonist phenoxybenzamine or the ß-receptor antagonist propranolol is ineffective at inhibiting estrogen/progesterone-induced lordosis in female rats.

## METHODS

### Animals

Sherman strain female rats weighing 200-250 g were obtained from Camm Research Co. (Wayne, NJ). Long Evans strain female rats weighing 200-250 g were obtained from Charles Rivers Laboratories (Wilmington, MA). All animals were maintained in a temperature- (21 ± 1° C) and light-(lights on between 2100 and 1100 h) controlled environment, and provided food (Wayne Lablox) and tap water ad libitum. For all experiments, rats were bilaterally ovariectomized under sodium pentobarbital anesthesia (30 mg/kg; i.p.), and one week later treated with estradiol benzoate (0.5 µg/0.1 ml/rat; i.m.) 72, 48, and 24 hours, and progesterone (500 µg/0.1 ml/rat; i.m.) 5 hours before a behavioral pre-test for

sexual receptivity. Only female rats attaining a lordosis quotient of 70 or greater in the behavioral pre-test, thereby demonstrating a response to exogenous hormone treatment, were used in the present studies.

**Drugs**

Estradiol benzoate (Sigma Chemical Co., St Louis, MO) and progesterone (Sigma) were dissolved in sesame oil. Propranolol hydrochloride (Sigma) was dissolved in 0.9% saline. Phenoxybenzamine (a gift from Smithkline Beecham, Swedeland, PA) was dissolved in 50% propylene glycol (Sigma).

**Treatment**

For each of the present studies, twenty female rats were randomly assigned to one of the following treatment groups and then randomly reassigned to receive a second treatment the following week. For all experiments, females were injected with estradiol benzoate (see appropriate figure legend for dose and time of treatment) and progesterone (500 μg/0.1 μl/rat; i.m.) 4 hours before behavioral testing.

To determine the effect of adrenergic antagonists on receptivity, estrogen (0.5 μg/rat; i.m. at 72, 48, and 24 hours before testing) and progesterone (500 μg/rat; i.m. at 4 hours before testing)-treated female rats of Sherman and Long Evans strains were injected with either propranolol (1, 4 or 20 mg/kg; i.p.) or its vehicle (0.9% saline; 1 ml/kg; i.p.); or with either phenoxybenzamine (1, 4 or 20 mg/kg; s.c.) or its vehicle (50% propylene glycol; 1 ml/kg; s.c.) 2 hours

prior to behavioral testing. Two different strains of rats were tested since results from preliminary studies were not in agreement with a previous report.

To determine if the effect of adrenergic antagonists on lordosis is dependant on estrogen treatment, Sherman strain female rats were injected with estrogen (either 0.125, 0.25, or 0.5 μg/rat; i.m. at 72, 48, and 24 hours before testing; or 4 or 10 μg/rat; i.m. at 48 hours before testing) and progesterone (500 μg/rat; i.m. at 4 hours before testing) followed by injections of either propranolol (4 mg/kg) or its vehicle (0.9% saline; 1 ml/kg; i.p.) 2 hours before behavioral testing, or in a separate experiment, phenoxybenzamine (4 mg/kg) or its vehicle (50% propylene glycol; 1 ml/kg; s.c.) 2 hours before behavioral testing.

**Behavioral Testing**

In all studies, female rats were tested for sexual receptivity by placing them with a sexually experienced male Long Evans rat which had been adapted to the testing arena (45x50x58 cm Plexiglas cage). Lordosis behavior was measured as a lordosis quotient (LQ) which is defined as the frequency of lordosis postures to ten mounts divided by ten and multiplied by 100 (LQ = number of lordosis responses/10 x 100). A mount was counted when the male palpated the female's flank with his forepaws and exhibited pelvic thrusting. Each test session was limited to 10 mounts.

**Statistics**

Lordosis quotients were analyzed with Kruskal-Wallis one-way analysis of variance by ranks followed by the Mann-Whitney U test for comparisons between two groups (Siegle, 1956). Differences were considered significant if the probability of error was less than 5%.

## RESULTS

As shown in Figure 1, injection of 1, 4, or 20 mg/kg of the ß-antagonist propranolol given 2 hours after progesterone treatment and 2 hours prior to behavioral testing failed to inhibit receptivity in either the Sherman or Long Evans strain of ovariectomized estrogen/progesterone-treated female rats. Likewise, in a similar experiment, injection of the $\alpha_1$-antagonist phenoxybenzamine (1, 4 or 20 mg/kg) failed to inhibit receptivity in either of these two strains (Figure 2). These results suggest that neither ß- nor $\alpha_1$-receptor blockade is sufficient to inhibit the high level of receptivity induced by estrogen and progesterone in these two strains of female rats.

To determine if the inability of either of the adrenergic receptor antagonists to block estrogen/progesterone induced receptivity was dependant on concentration or duration of exposure to estrogen, ovariectomized females were primed with various concentrations of estrogen that were administered

**Figure 1** Effects of propranolol on lordosis in ovariectomized, estrogen/progesterone-treated female rats of two different strains. Sherman and Long Evans strain female rats were injected with either propranolol (1, 4 or 20 mg/kg; i.p.) or its vehicle (0.9% saline; 1 ml/kg) 2 hours before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 10 determinations of sexual receptivity (lordosis quotient) in Sherman or Long Evans strain female rats treated with either propranolol (solid columns) or its vehicle (open columns).

Figure 1

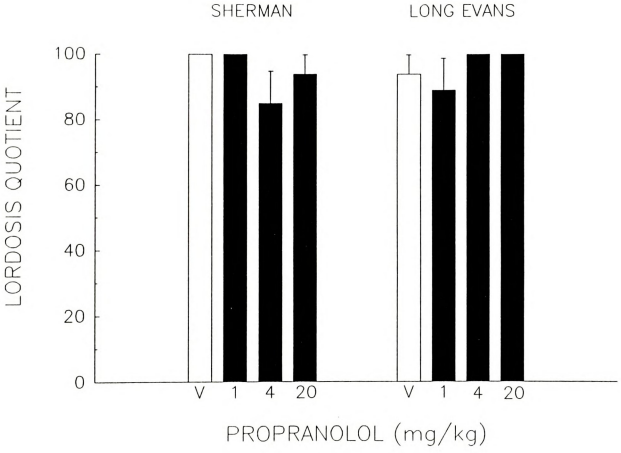
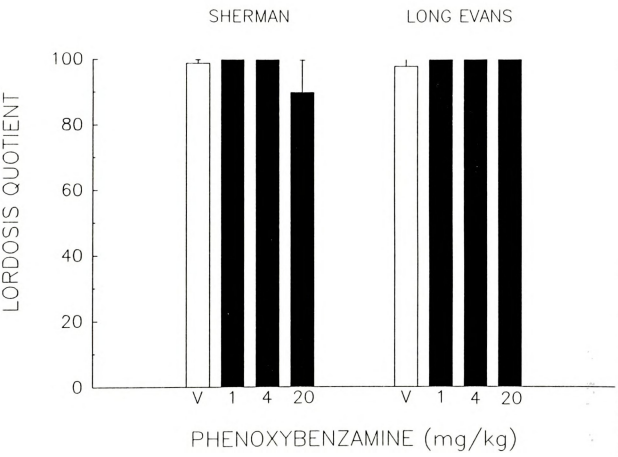

SHERMAN
LONG EVANS
LORDOSIS QUOTIENT
100
80
60
40
20
0
V
1
4
20
V
1
4
20
PROPRANOLOL (mg/kg)

**Figure 2** Effects of phenoxybenzamine on lordosis in ovariectomized, estrogen/progesterone-treated female rats of two different strains. Sherman and Long Evans strain female rats were injected with either phenoxybenzamine (1, 4 or 20 mg/kg; s.c.) or its vehicle (50% propylene glycol; 1 ml/kg) 2 hours before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 9-10 determinations of sexual receptivity (lordosis quotient) in Sherman or Long Evans strain female rats treated with either phenoxybenzamine (solid columns) or its vehicle (open columns).

Figure 2


SHERMAN
LONG EVANS
LORDOSIS QUOTIENT
100
80
60
40
20
0
V 1 4 20
V 1 4 20
PHENOXYBENZAMINE (mg/kg)

over various time schedules. As shown in Figure 3, estrogen/progesterone-treated female rats given 0.125 μg of estrogen 72, 48 and 24 hours before testing were less receptive than females treated with the higher doses of estrogen, and propranolol failed to inhibit lordosis as compared to the vehicle-treated females for any of the estrogen-treatment groups. Likewise as shown in Figure 4, estrogen/progesterone-treated females were less receptive when primed with lower doses of estrogen, and phenoxybenzamine failed to inhibit lordosis in any of the estrogen-treatment groups. These results indicate that systemic administration of the adrenergic antagonists propranolol and phenoxybenzamine does not block estrogen/progesterone induced lordosis regardless of estrogen-treatment and level of receptivity.

## DISCUSSION

The role of adrenergic receptor subtypes in mediating hormone induced receptivity in female rats has remained unclear. In contrast to the results of the present study, Fernández-Guasti and coworkers found that systemic injection of the $\alpha_1$-antagonists phenoxybenzamine or prazosin, or the ß-antagonist propranolol inhibited lordosis in estrogen/progesterone-treated females when given 2 hours after progesterone injection (Fernández-Guasti, at al., 1985a). These results suggest that either $\alpha_1$- or ß-receptor blockade is sufficient to inhibit lordosis and that activation of both

**Figure 3** Effects of propranolol on lordosis in ovariectomized estrogen/progesterone-treated female rats given various treatments of estrogen. Females were treated with either 0.125 μg, 0.25 μg, or 0.5 μg/rat; i.m. of estradiol benzoate at 72, 48 and 24 hours, or either 4 μg or 10 μg/rat; i.m. of estradiol benzoate at 48 hours before behavioral testing. All estrogen-treated females received progesterone (500 μg/rat; i.m.) 4 hours before behavioral testing. All groups were injected with either propranolol (4 mg/kg) or its vehicle (0.9% saline; 1 ml/kg; i.p.) 2 hours before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations of sexual receptivity (lordosis quotient) in vehicle- (open column) or propranolol-treated (solid column) rats.

Figure 3

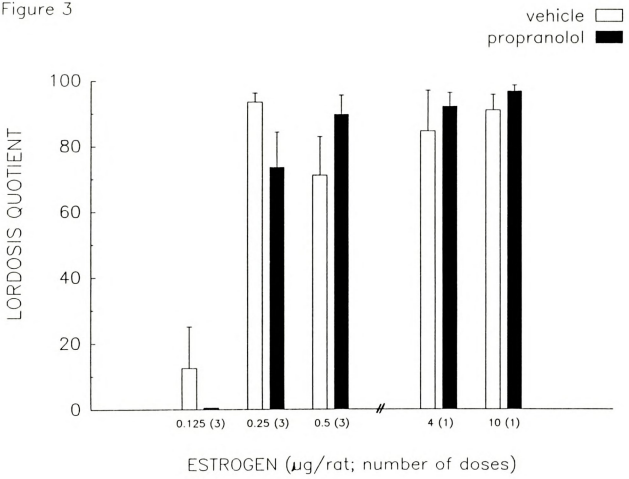
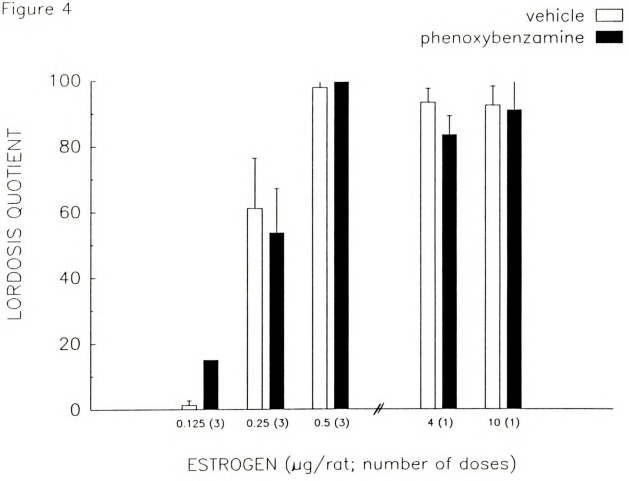

vehicle
propranolol
LORDOSIS QUOTIENT
100
80
60
40
20
0
0.125 (3)
0.25 (3)
0.5 (3)
4 (1)
10 (1)
ESTROGEN (µg/rat; number of doses)

**Figure 4** Effects of phenoxybenzamine on lordosis in ovariectomized estrogen/progesterone-treated female rats given various treatments of estrogen. Females were treated with either 0.125 μg, 0.25 μg, or 0.5 μg/rat; i.m. of estradiol benzoate at 72, 48 and 24 hours, or either 4 μg or 10 μg/rat; i.m. of estradiol benzoate at 48 hours before behavioral testing. All estrogen-treated females received progesterone (500 μg/rat; i.m.) 4 hours before behavioral testing. All groups were injected with either phenoxybenzamine (4 mg/kg) or its vehicle (50% propylene glycol; 1 ml/kg; s.c.) 2 hours before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 6-8 determinations of sexual receptivity (lordosis quotient) in vehicle- (open column) or phenoxybenzamine-treated (solid column) rats.

Figure 4


vehicle
phenoxybenzamine
LORDOSIS QUOTIENT
100
80
60
40
20
0
0.125 (3)
0.25 (3)
0.5 (3)
4 (1)
10 (1)
ESTROGEN (μg/rat; number of doses)

receptor types are required for receptivity. In the present experiment, however, neither injection of the $\alpha_1$-antagonist phenoxybenzamine or the ß-antagonist propranolol into estrogen/progesterone-treated female rats inhibited lordosis, regardless of the female strain used. The failure to decrease the occurrence of lordosis was not dependant on estrogen dose or the level of receptivity in females. These results are in agreement with Davis and Kohl, who also failed to see an effect of phenoxybenzamine on lordosis (Davis and Kohl, 1977). Interestingly, these investigators found that systemic injections of the $\alpha_2$-agonist, clonidine, inhibited lordosis in estrogen/progesterone-treated females (possibly as a result of inhibiting presynaptic NE release). Further, they found that this inhibition was blocked by pretreatment with the $\alpha_2$-antagonist yohimbine. Variations in the results from various laboratories might possibly be due to an inconsistency of systemic injections to provide the required concentration of antagonist at the sites of its action. It is unlikely that the conflicting results are due to female rat strain differences.

## EXPERIMENT 2: INJECTION OF NE INTO THE VMN FAILS TO FACILITATE LORDOSIS IN ESTROGEN-TREATED FEMALE RATS

Various lines of evidence lend strong support to the notion that the VMN is an important part of the brain circuitry controlling hormone-mediated sexual behavior. There are also data which indicate that NE is in some way involved in these pathways. Conceivably, NE could be acting in the VMN to mediate the effects of estrogen and progesterone on lordosis since NE is found to increase in this region during sexually receptive behavior in hormone-treated female rats (Vathy and Etgen, 1989).

In the following study, unreceptive estrogen-primed female rats were used to determine if increases in NE in the VMN could facilitate lordosis. The females used for this study were treated with low doses of estrogen (without progesterone treatment) and would not display receptivity when exposed to a male. The results indicate that injection of NE into the area of the VMN only, is not sufficient to induce receptivity in female rats primed only with low doses of estrogen.

### METHODS

#### Animals

Sherman strain female rats weighing 200-250 g were obtained from Camm Research Co. (Wayne, NJ). All animals were maintained in a temperature- (21 ± 1° C) and light- (lights on between 2100 and 1100 h) controlled environment, and provided

food (Wayne Lablox) and tap water ad libitum. Females were bilaterally ovariectomized under sodium pentobarbital anesthesia (30 mg/kg; i.p.), and one week later treated with estradiol benzoate (0.5 µg/0.1 ml/rat; i.m.) 72, 48, and 24 hours, and progesterone (500 µg/0.1 ml/rat; i.m.) 5 hours before a behavioral pre-test for sexual receptivity. Only females attaining a lordosis quotient of 70 or greater in the behavioral pre-test, thereby demonstrating a response to exogenous hormone treatment, were used in the present studies.

**Drugs**

Estradiol benzoate (Sigma Chemical Co., St Louis, MO) and progesterone (Sigma) were dissolved in sesame oil. Norepinephrine hydrochloride (Sigma) was dissolved in artificial CSF immediately before use.

**Intracerebral Injections into the VMN**

Ten animals receiving VMN injections of norepinephrine or its vehicle were implanted with stainless steel guide cannula 7 days prior to the experiment. Rats were anesthetized with sodium pentobarbital (30 mg/kg; i.p.) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set at the horizontal plane (König and Klippel, 1963). Bilateral 23-gauge stainless-steel guide cannulae were implanted 2.3 mm posterior to bregma, ±0.8 mm from the midline and 7.5 mm below dura, and anchored to the skull with stainless-steel screws and dental cement. On the day of the experiment, fifteen minutes prior to behavioral

testing, norepinephrine (200 ng/side) or its artificial CSF vehicle (0.5 µl/side) were injected bilaterally using a 10 µl Hamilton microsyringe connected to a 30 gauge stainless-steel injector which protruded 1 mm beyond the tip of the cannula guide and into the VMN. Following completion of the study, animals were anesthetized with sodium pentobarbital (60 mg/kg; i.p.) and perfused with 0.9% saline followed by a 10% formalin solution. The brains were removed and frontal sections (50 µm) through the VMN were prepared using a microtome with a CO2 freezing stage. Mounted brain sections were stained with cresyl violet and examined for cannulae placement in the VMN. Only data from females with cannulae placed in the VMN were used for statistical analyses.

**Behavioral Testing**

In the present study, ten VMN-cannulated female rats were randomly assigned to one of two treatment groups (NE or its vehicle) and, one week later, reassigned to the second treatment group. This design allowed each female to be tested twice, receiving both treatments. All females were injected with a low dose of estradiol benzoate (0.175 µg/0.1 ml/rat; i.m.) 72, 48, and 24 hours before behavioral testing. Female rats were tested for sexual receptivity by placing them with a sexually experienced male Long Evans rat which had been adapted to the testing arena (45x50x58 cm Plexiglas cage). Lordosis behavior was measured as a lordosis quotient (LQ) which is defined as the frequency of lordosis postures to ten

mounts divided by ten and multiplied by 100 (LQ = number of lordosis responses/10 x 100). A mount was counted when the male palpated the female's flank with his forepaws and exhibited pelvic thrusting. Each test session was limited to 10 mounts.

**Statistics**

Lordosis quotients were analyzed using the Mann-Whitney U test for comparisons between two groups (Siegle, 1956). Differences were considered significant if the probability of error was less than 5%.

## RESULTS

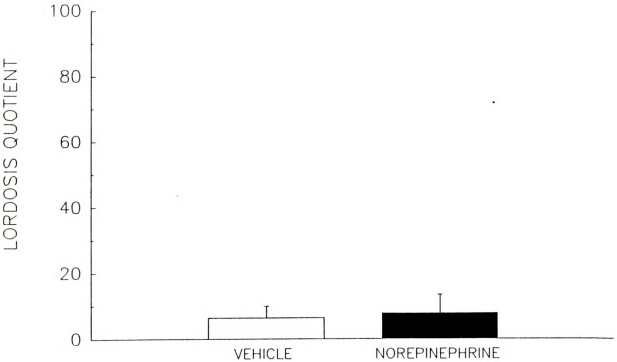
As shown in Figure 5, bilateral injections of NE (400 ng/rat; 200 ng/side) into the VMN failed to facilitate receptivity in ovariectomized females rats treated with low doses of estrogen as compared to the non-receptive, vehicle-treated controls. These results indicate that injections of NE in the area of the VMN are not sufficient to facilitate receptivity in ovariectomized females treated with low doses of estrogen.

## DISCUSSION

Several lines of evidence suggest that NE transmission in the VMN is important for the display of lordosis. This may, however, only be a minor portion of the whole circuitry responsible for expression of sexual behavior. NE has been

**Figure 5** Effect of bilateral injections of NE into the ventromedial nucleus (VMN) on lordosis in ovariectomized, estrogen-treated female rats. Rats were injected with either NE (200 ng/side) or its vehicle (artificial CSF; 0.5 $\mu$l/side) into the VMN 15 minutes before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 9 determinations of sexual receptivity (lordosis quotient) in vehicle- (open column) or NE-treated (solid column) rats.

Figure 5



shown to increase in the area of the VMN of estrogen/progesterone-treated receptive female rats, although it is not known if NE is increasing in other brain regions important for lordosis as well (Vathy and Etgen, 1989). In contrast to the findings of the present study, NE injections into the VMN have been shown to facilitate lordosis in estrogen only-treated female rats (Foreman and Moss, 1978; Fernández-Guasti, et al., 1985b). This effect was blocked by systemic injection of either an $\alpha_1$- or ß-antagonist (Fernández-Guasti, et al., 1985b), suggesting that NE action may be required at both $\alpha_1$- and ß-receptors in the area of the VMN.

In the present study, injections of NE into the VMN failed to facilitate lordosis in estrogen-treated female rats. The reason for differences between the present study and other investigators findings is unclear but might be due to several factors. There could be variations in the sites of injection between experiments or differences in NE concentration available at the sites, even though doses of NE were comparable. In other studies, leakage of NE from the VMN to other important brain areas cannot be ruled out. Although NE may act at different adrenergic receptor subtypes to inhibit or facilitate lordosis, these results suggest that exogenous increases of NE in the VMN alone is insufficient to induce lordosis in ovariectomized female rats treated with low doses of estrogen.

**EXPERIMENT 3: NE IN EITHER THE VMN OR MPN IS NOT ESSENTIAL FOR LORDOSIS IN ESTROGEN/PROGESTERONE-TREATED FEMALE RATS**

Further evidence to support a role of NE in the control of lordosis is demonstrated by various lesion studies. Electrolytic- and neurotoxin-induced lesions of the ventral noradrenergic bundle (VNAB) deplete NE concentrations in the basal forebrain and disrupt lordosis in ovariectomized estrogen/progesterone-treated female rats (Hansen, et al., 1981). It is not known, however, if loss of NE in the VMN, MPN, or other regions following these lesions is responsible for the reduction in lordosis.

The following experiments were performed in an attempt to determine the site of NE action in the control of lordosis. The effects of selective neurotoxin (5-ADMP)-induced lesions of noradrenergic neurons terminating in the VMN, MPN, or medial amygdala (mAMY) on lordosis were examined in ovariectomized, estrogen/progesterone-treated female rats. The results reveal that while neurotoxin-induced depletion of NE in the basal forebrain inhibits lordosis, selective NE depletion in either the VMN, MPN, or mAMY is not accompanied by a loss of lordosis. These data suggest that noradrenergic neurons terminating in any one of these nuclei are not essential, by themselves, for the induction of sexual receptivity in ovariectomized female rats by gonadal steroids.

## METHODS

### Animals

Sherman strain female rats weighing 200-250 g were obtained from Camm Research Co. (Wayne, NJ). All animals were maintained in a temperature- (21 ± 1° C) and light- (lights on between 2100 and 1100 h) controlled environment, and provided food (Wayne Lablox) and tap water ad libitum. For all experiments, rats were bilaterally ovariectomized under sodium pentobarbital anesthesia (30 mg/kg; i.p.), and one week later treated with estradiol benzoate (0.5 μg/0.1 ml/rat; i.m.) 72, 48, and 24 hours, and progesterone (500 μg/0.1 ml/rat; i.m.) 5 hours before a behavioral pre-test for sexual receptivity. Only female rats attaining a lordosis quotient of 70 or greater in the behavioral pre-test, thereby demonstrating a response to exogenous hormone treatment, were used in the present studies.

### Drugs

Estradiol benzoate (Sigma Chemical Co., St Louis, MO) and progesterone (Sigma) were dissolved in sesame oil. Fluoxetine hydrochloride (Eli Lilly and Co., Indianapolis, IN) was dissolved in 0.9% saline. 5-Amino-2,4,dihydroxy-α-methylphenylethylamine dihydrobromide (5-ADMP; synthesized by Dr. John R. Palmer of The Upjohn Co., Kalamazoo, MI) was dissolved in 0.3% saline containing 0.1% ascorbic acid. Phenylephrine hydrochloride (Sigma) was dissolved in artificial CSF. Drugs were administered as indicated in the

legends of the appropriate table and figures; doses of fluoxetine, 5-ADMP and phenylephrine were calculated as free base.

**Neurochemical Lesions of the VNAB**

Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.) with the incisor bar set 3 mm below the horizontal plane. The needle of a 5 µl Hamilton syringe was inserted into the VNAB at coordinates A 0.0 mm, L ±1.3 mm, V -6.8 mm from dura (15), and bilateral injections of either 5-ADMP (8 µg/side) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 µl/side) were made over a 1 minute period. The needle remained in the brain for an additional 10 minutes after injection to reduce the reflux of the neurotoxin back up the needle track. One hour prior to administration of 5-ADMP rats were injected with fluoxetine (10 mg/kg; s.c.) to prevent the uptake of neurotoxin into 5-hydroxytryptaminergic neurons. Rats were allowed to recover for 7 days following surgery before behavioral testing was performed.

**Neurochemical Lesions of the VMN, MPN and mAMY**

Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set 2.4 mm below the horizontal plane (König and Klippel, 1963). For the VMN, the needle of a 5 µl Hamilton syringe was inserted at coordinates A 4.4 mm, L ±0.7 mm, V -8.8 mm from dura, and bilateral

injections of either 5-ADMP (2 μg/side) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/side) were made over a 1 minute period. For the MPN, the needle of a 5 μl Hamilton syringe was inserted at coordinates A 6.8 mm, L ±0.7 mm, V -7.7 mm from dura, and two bilateral injections of either 5-ADMP (2 μg/site/side) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/site/side) were made over a 2 minute period by injecting for the first minute at the most ventral position (7.7 mm below dura) and then raising the needle to inject more dorsally (7.0 mm below dura) for the final minute. For the mAMY, the needle of a 5 μl Hamilton syringe was inserted at coordinates A 2.1 mm, L ±3.2 mm, V -8.0 mm from dura, and bilateral injections of either 5-ADMP (3 μg/side) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/side) were made over a 1 minute period. The needle remained in the brain for an additional 10 minutes after the final injection to reduce the reflux of the neurotoxin back up the needle track. One hour prior to injection of 5-ADMP rats were injected with fluoxetine (10 mg/kg; s.c.) to prevent uptake of neurotoxin into 5-hydroxytryptamine (5HT) neurons. Rats were allowed to recover for 7 days following surgery before behavioral testing was performed.

**Lateral Ventricular Cannulation**

Animals receiving intracerebroventricular (i.c.v.) injections of phenylephrine or its vehicle were implanted with

a stainless steel guide cannula into a lateral cerebral ventricle 7 days prior to the experiment. Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set 2.4 mm below the horizontal plane (König and Klippel, 1963). A 23-gauge stainless-steel guide cannula was implanted such that the tip was 1.4 mm lateral to bregma and 3.2 mm below dura, and anchored to the skull with stainless-steel screws and dental cement. On the day of the experiment, phenylephrine or its artificial CSF vehicle were injected in a volume of 5 µl with a 10 µl Hamilton microsyringe connected to a 30 gauge stainless-steel injector which protruded 1 mm beyond the tip of the cannula guide and into the lateral ventricle.

**Intracerebral Injections into Hypothalamic Nuclei**

Animals receiving intracerebral (i.c.) injections of phenylephrine or its vehicle into the VMN or MPN were implanted with stainless steel guide cannula 7 days prior to the experiment. Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set 2.4 mm below the horizontal plane (König and Klippel, 1963). For the VMN, bilateral 23-gauge stainless steel cannula were implanted ±0.7 mm from midline, 2.6 mm posterior to bregma and 8.8 mm below dura. For the MPN, bilateral 23-gauge stainless steel cannula were implanted ±0.7 mm from midline, 2.4 mm posterior

to bregma and 7.4 mm below dura. Phenylephrine (1 μg/side) or its artificial CSF vehicle (0.3 μl/side) were injected bilaterally into the VMN or MPN with a 10 μl Hamilton microsyringe connected to a 30 gauge stainless-steel injector 30 minutes prior to behavioral testing.

**Behavioral Testing**

For all experiments, females were injected with estradiol benzoate (0.5 μg/0.1 ml/rat; i.m.) 72, 48, and 24 hours, and progesterone (500 μg/0.1 ml/rat; i.m.) 5 hours before behavioral testing. Female rats were tested for sexual receptivity by placing them with a sexually experienced male Long Evans rat which had been adapted to the testing arena (45x50x58 cm Plexiglas cage). Lordosis behavior was measured as a lordosis quotient (LQ) which is defined as the frequency of lordosis postures to ten mounts divided by ten and multiplied by 100 (LQ = number of lordosis responses/10 x 100). A mount was counted when the male palpated the female's flank with his forepaws and exhibited pelvic thrusting. Each test session was limited to 10 mounts.

**Tissue Dissection and Neurochemical Analyses**

Within one hour following behavioral testing, animals were decapitated and brains were removed from the skull and frozen on aluminum foil placed directly over dry ice. Frontal brain sections (600 μm) beginning approximately at 9220 μm (König and Klippel, 1963) were prepared in a cryostat (-9°C), and the VMN and MPN were dissected from these sections

according to a modification (Lookingland and Moore, 1985) of the method of Palkovits (Palkovits, 1973). Tissues samples were placed in 60 µl of 0.1 M phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at -20°C until assayed.

On the day of the assay tissue samples were thawed, sonicated for 3 s (Sonicator Cell Disruptor, Heat Systems-Ultrasonic, Plainview, NY), and centrifuged for 30 s in a Beckman 152 Microfuge. NE, dopamine (DA) and 5HT concentrations in supernatants were measured by high performance liquid chromatography with electrochemical detection as described previously (Chapin, et al., 1986). Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein (Lowry, et al., 1951).

**Statistics**

Statistical analyses of monoamine concentrations were performed using the Student's t test to compare differences between two groups, and one-way analysis of variance followed by the least significant difference test for the comparison of multiple groups (Steel and Torrie, 1960). Lordosis quotients were analyzed with Kruskal-Wallis one-way analysis of variance by ranks followed by the Mann-Whitney U test for comparisons between two groups (Siegle, 1956). Differences were considered significant if the probability of error was less than 5%.

## RESULTS

As shown in Table 1, NE concentrations were significantly reduced to 35% of control in the VMN and to 30% of control in the MPN 7 days following bilateral injections of 5-ADMP into the VNAB. In contrast, 5-ADMP had no effect on the concentrations of DA or 5HT in these brain regions. 5-ADMP injections into the VNAB significantly reduced lordosis quotients in ovariectomized, estrogen/progesterone-treated female rats (Figure 6), and this effect was reversed by i.c.v. administration of the $\alpha_1$-adrenergic receptor agonist phenylephrine (Figure 7). Taken together these results suggest that neurotoxin-induced disruption of noradrenergic neurons is associated with a deficit in sexual receptivity in female rats.

To determine if the reduction in sexual receptivity following 5-ADMP-induced lesions of the VNAB resulted from loss of noradrenergic neuronal projections to the VMN or MPN, lordosis quotients were determined in ovariectomized, estrogen/progesterone-treated rats in which noradrenergic terminals in these hypothalamic nuclei were selectively lesioned. As shown in Figure 8, injection of 5-ADMP directly into the VMN reduced NE concentrations in the VMN to 17% of control, but failed to alter lordosis quotients as compared with vehicle-treated controls. Similarly, direct injection of 5-ADMP into the MPN reduced NE concentrations in the MPN to 17% of control, but failed to alter lordosis quotients as

**Table 1** Effect of bilateral injections of 5-ADMP into the ventral noradrenergic bundle (VNAB) on amine concentrations in the ventromedial nucleus (VMN) and medial preoptic nucleus (MPN) of ovariectomized, estrogen/progesterone-treated female rats.

| | | Amine Concentration (ng/mg protein) | | |
|---|---|---|---|---|
| | | **NE** | **DA** | **5HT** |
| **VMN** | vehicle | 14.5 ± 1.1§ | 1.2 ± 0.1 | 6.1 ± 0.3 |
| | 5-ADMP# | 5.1 ± 0.7* | 1.1 ± 0.1 | 6.4 ± 0.4 |
| **MPN** | vehicle | 24.3 ± 1.5 | 6.7 ± 2.7 | 10.4 ± 0.5 |
| | 5-ADMP | 7.2 ± 0.7* | 5.5 ± 2.2 | 10.3 ± 0.5 |

§ values represent the means ± 1 S.E.M. of 9-11 determinations.

# rats were injected with 5-ADMP or its vehicle into the VNAB and killed by decapitation 7 days later.

* values for 5-ADMP-treated rats that are significantly different from vehicle-treated controls ($p<0.05$).

**Figure 6** Effects of bilateral injections of 5-ADMP into the ventral noradrenergic bundle (VNAB) on lordosis in ovariectomized, estrogen/progesterone-treated female rats. Rats were injected with either 5-ADMP (8 μg/side; i.c.) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/side) into the VNAB 7 days prior to behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 9-11 determinations of sexual receptivity (lordosis quotient) in vehicle- (open column) or 5-ADMP-treated (solid column) rats. *, values for 5-ADMP-treated rats that are significantly different from vehicle-treated controls ($p<0.05$).

Figure 6

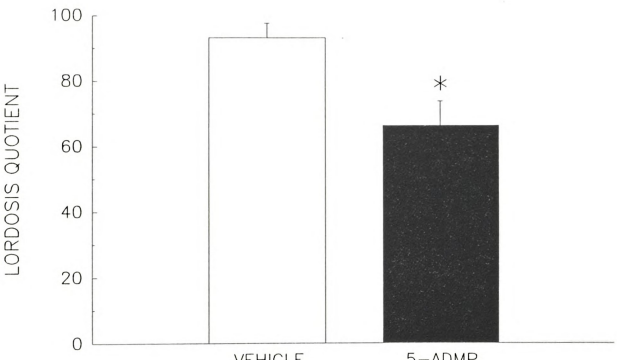


**Figure 7** Effect of phenylephrine on lordosis in VNAB-lesioned ovariectomized, estrogen/progesterone-treated female rats. VNAB-lesioned rats were injected with either phenylephrine (1 μg/rat; i.c.v.) or its vehicle (artificial CSF; 5 μl/rat; i.c.v.) thirty minutes before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 6-8 determinations of sexual receptivity (lordosis quotient) in sham- (open column) or VNAB-lesioned (solid columns) rats. *, values for VNAB-lesioned rats that are significantly different from sham-lesioned controls ($p<0.05$).

Figure 7

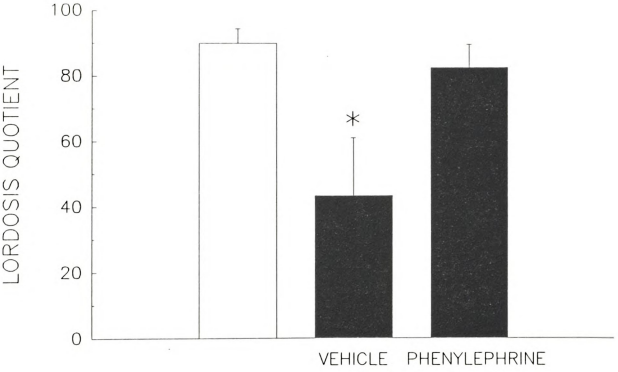
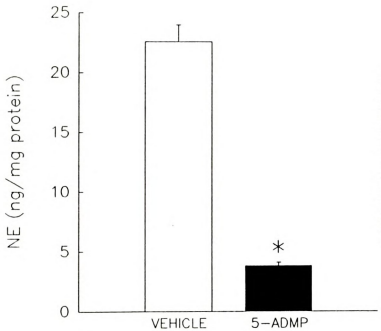
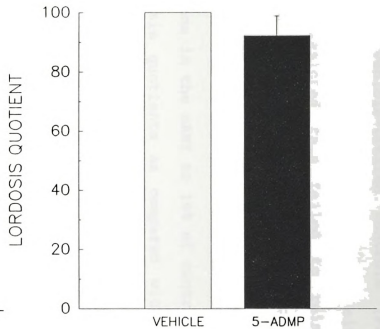


**Figure 8** Effects of bilateral injections of 5-ADMP into the ventromedial nucleus (VMN) on NE concentrations in the VMN and lordosis in ovariectomized, estrogen/progesterone-treated female rats. Rats were injected with either 5-ADMP (2 μg/side; i.c.) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/side) into the VMN 7 days prior to behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 9 determinations of NE concentrations (ng/mg protein) in the VMN (left panel) or sexual receptivity (lordosis quotient; right panel) in vehicle- (open column) or 5-ADMP-treated (solid column) rats. *, values for 5-ADMP-treated rats that are significantly different from vehicle-treated controls ($p<0.05$).

Figure 8


25
20
15
10
5
0
NE (ng/mg protein)
*
VEHICLE
5-ADMP


100
80
60
40
20
0
LORDOSIS QUOTIENT
VEHICLE
5-ADMP

compared with vehicle-treated controls (Figure 9). In addition, injection of phenylephrine into either the VMN or MPN of VNAB-lesioned rats failed to reinstate lordosis quotients to the levels determined in the sham-lesioned controls (Figure 10). As was the case for the VMN and MPN, direct injection of 5-ADMP into the mAMY reduced NE concentrations in the mAMY to 19% of control, but failed to alter lordosis quotients as compared with vehicle-treated controls (Figure 11). Taken together these results indicate that noradrenergic neurons terminating in either the VMN, MPN, or mAMY are not by themselves necessary for sexual receptivity in ovariectomized, estrogen/progesterone-treated female rats.

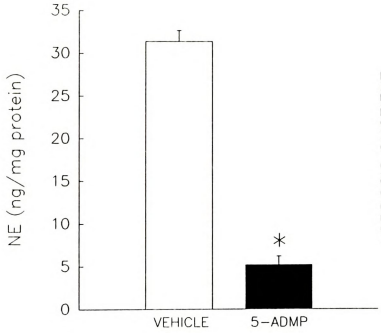
## DISCUSSION

Neurons in the VMN and MPN are believed to play an important role in the mediation of hormone-induced sexually receptive behaviors in female rats. However, the involvement of NE in these and other lordosis relevant regions are unclear. In the past it has been thought that NE (under the influence of steroid hormones) acts on neurons of a specific brain region within the neural circuit responsible for sexual behavior.

In the present study, disruption of subcoeruleus noradrenergic neurons in the VNAB following i.c. administration of the selective noradrenergic neurotoxin 5-ADMP (Jarry, et al., 1986) resulted in the loss of lordosis

**Figure 9** Effects of bilateral injections of 5-ADMP into the medial preoptic nucleus (MPN) on NE concentrations in the MPN and lordosis in ovariectomized, estrogen/progesterone-treated female rats. Rats were injected with either 5-ADMP (4 μg/side; i.c.) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.6 μl/side) into the MPN 7 days prior to behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 10-11 determinations of NE concentrations (ng/mg protein) in the MPN (left panel) or sexual receptivity (lordosis quotient; right panel) in vehicle- (open column) or 5-ADMP-treated (solid column) rats. *, values for 5-ADMP-treated rats that are significantly different from vehicle-treated controls ($p<0.05$).

Figure 9


NE (ng/mg protein)
35
30
25
20
15
10
5
0
*
VEHICLE
5-ADMP

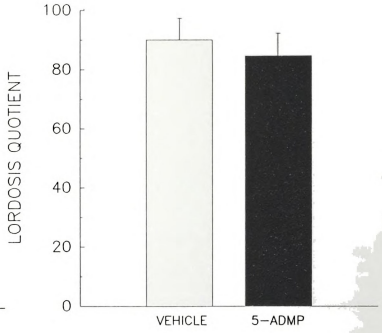
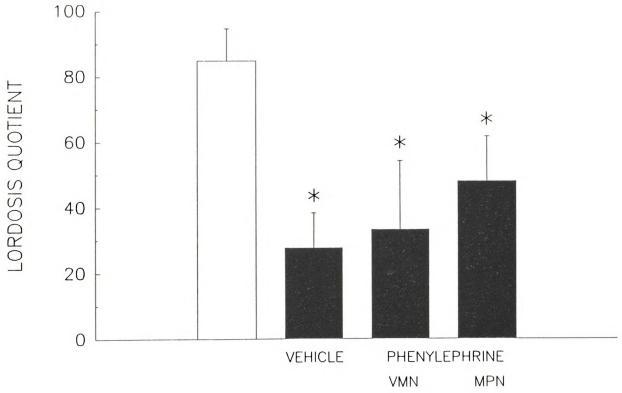

LORDOSIS QUOTIENT
100
80
60
40
20
0
VEHICLE
5-ADMP

**Figure 10** Effects of administration of phenylephrine into the ventromedial nucleus (VMN) or medial preoptic nucleus (MPN) on lordosis in VNAB-lesioned ovariectomized, estrogen/progesterone-treated female rats. VNAB-lesioned rats were injected with either phenylephrine (1 μg/side) or its vehicle (artificial CSF; 0.3 μl/side) into either the VMN or MPN 30 minutes before behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 6-14 determinations of sexual receptivity (lordosis quotient) in sham- (open column) or VNAB-lesioned (solid columns) rats. *, values for VNAB-lesioned rats that are significantly different from sham-lesioned controls ($p<0.05$).

Figure 10


100
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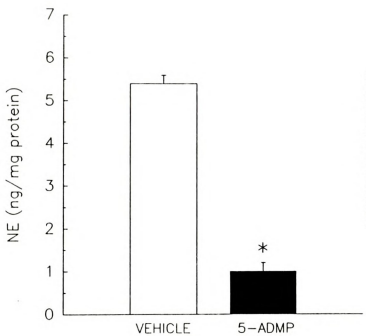
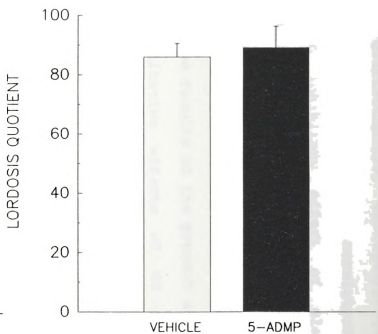
LORDOSIS QUOTIENT
*
*
*
VEHICLE
PHENYLEPHRINE
VMN
MPN

**Figure 11** Effects of bilateral injections of 5-ADMP into the medial amygdala (mAMY) on NE concentrations in the mAMY and lordosis in ovariectomized, estrogen/progesterone-treated female rats. Rats were injected with either 5-ADMP (2 μg/side; i.c.) or its vehicle (0.3% saline containing 0.1% ascorbic acid; 0.3 μl/side) into the mAMY 7 days prior to behavioral testing. Columns represent the means and vertical lines 1 S.E.M. of 10 determinations of NE concentrations (ng/mg protein) in the mAMY (left panel) or sexual receptivity (lordosis quotient; right panel) in vehicle- (open column) or 5-ADMP-treated (solid column) rats. *, values for 5-ADMP-treated rats that are significantly different from vehicle-treated controls ($p<0.05$).

Figure 11


NE (ng/mg protein)
7
6
5
4
3
2
1
0
*
VEHICLE
5-ADMP


LORDOSIS QUOTIENT
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VEHICLE
5-ADMP

behavior in ovariectomized, estrogen/progesterone-treated female rats. These results are consistent with previous reports of an inhibitory effect of electrolytic- and neurotoxin-induced lesion of the VNAB on lordosis behavior (Hansen, et al., 1981), and indicate that subcoeruleus noradrenergic neurons are important for the control of sexual receptivity. The results of the present study also indicate that the facilitative effects of NE on female sexual receptivity are mediated by $\alpha_1$-adrenergic receptors since i.c.v. injection of the $\alpha_1$-adrenergic receptor agonist phenylephrine restores lordosis in VNAB-lesioned female rats, although the involvement of ß-adrenergic receptors can not be ruled out.

A number of reports have implicated either the VMN or MPN as possible sites of action of NE on lordosis in female rats. In the present study, however, depletion of NE in either the VMN or MPN did not reduce lordosis in ovariectomized, estrogen/progesterone-treated female rats. In addition, injection of the $\alpha_1$-adrenergic receptor agonist phenylephrine into either the VMN or MPN of hormone-treated females with VNAB-lesions failed to restore lordosis. These results suggest that noradrenergic neurons in the VMN or MPN alone are not sufficient to induce lordosis in ovariectomized, estrogen/progesterone-treated female rats.

Another region of the brain reported to be important for sexual receptivity in female rats is the corticomedial

amygdala, and disruption of noradrenergic innervation to this brain region occurs following VNAB lesions (Fallon, et al., 1978). The amygdala contains neurons that concentrate labeled estrogen (Pfaff and Keiner, 1973; Stumpf, et al., 1975), and electrolytic lesions in the anterior portion of the corticomedial amygdala disrupts lordosis, while electrochemical stimulation of this region facilitates lordosis in ovariectomized, steroid-treated female rats (Masco and Carrier, 1980). However, in the present study, depletion of NE in the region of the mAMY, as in either the VMN or MPN, failed to prevent hormone induced sexual receptivity. Alternatively, NE may play a neuromodulatory role in facilitating lordosis, and noradrenergic innervation to multiple brain nuclei (possibly including the VMN, MPN, or mAMY) may be required for full expression of sexual receptivity in female rats.

In conclusion, although previous reports indicate that the VMN and MPN are important hypothalamic regions for the control of lordosis, the results of the present study indicate that noradrenergic neurons terminating in either one of these regions are, in and of themselves, not essential for gonadal steroid induction of sexual receptivity in ovariectomized female rats.

## GENERAL DISCUSSION

Sexually receptive behavior in female rats requires estrogen and progesterone for full expression (Whalen, 1974). Although multiple sites of action for estrogen and progesterone probably exist, evidence suggests that these hormones exert a major facilitatory effect on female sexual receptivity through action on neurons located in the VMN and MPN of the hypothalamus. Neurons located in these regions concentrate labeled estrogen (Pfaff and Keiner, 1973; Stumpf, et al., 1975) and progesterone (MacLusky and McEwen, 1980; Parsons, et al., 1982; DonCarlos and Morrell, 1990), and ovariectomized female rats become receptive when given VMN implants of estrogen and systemic injections of progesterone (Rubin and Barfield, 1980). Conversely, implants of antiestrogens into the VMN block the stimulatory effects of estrogen/progesterone treatment on receptivity in ovariectomized female rats (Meisel, et al., 1987). In addition, electrolytic lesions of the VMN abolish lordosis (Pfaff and Sakuma, 1979; Mathews, et al., 1983). Electrolytic lesions of the dorsal region of the MPN reduce lordosis in ovariectomized female rats given systemic injections of estrogen and progesterone (Leedy, 1984), and in ovariectomized female rats implanted with estrogen in the VMN and injected systemically with progesterone (Bast, et al., 1987). Thus, while neurons in the VMN and MPN are implicated in mediating gonadal steroid-induced sexually receptive behaviors, the

identity of the neurotransmitters involved in this action are less well defined.

There are several lines of evidence to suggest that NE is involved in the brain circuitry controlling hormone-mediated sexual receptivity. Ovarian hormones have been shown to influence the transmission of noradrenergic neurons by effecting uptake and release (Janowsky and Davis, 1970; Vathy and Etgen 1988), firing rates (Kaba, et al., 1983), and NE turnover (Renner, et al., 1986). In addition, estrogen-concentrating noradrenergic neurons located in the pons-medulla project to basal forebrain areas which also contain estrogen-concentrating neurons. Results from Experiment 3 demonstrate that selective neurotoxic lesions of the subcoeruleus noradrenergic neurons which ascend in the VNAB, deplete NE in basal forebrain regions and also result in the loss of lordosis behavior in ovariectomized, estrogen/progesterone-treated female rats. These results are consistent with previous findings that electrolytic- and neurotoxin-induced lesions of the VNAB inhibit lordosis behavior (Hansen, et al., 1981). Together, the results of these studies give strong evidence that subcoeruleus noradrenergic neurons are important for the control of sexual receptivity.

The results from Experiment 3 also suggest that the facilitative effects of NE on female sexual receptivity are mediated by $\alpha_1$-adrenergic receptors, since lordosis behavior

was restored in VNAB-lesioned female rats following i.c.v. injection of the $\alpha_1$ receptor agonist phenylephrine. Unfortunately, most studies performed in an attempt to determine the adrenergic receptor subtype involved in the control of lordosis have yielded inconsistent information. The results from Experiment 1 indicate that blocking $\alpha_1$ receptors in estrogen/progesterone-treated female rats with systemic injections of the $\alpha_1$-antagonist phenoxybenzamine, does not inhibit receptivity. The failure of phenoxybenzamine, as well as the ß-receptor antagonist propranolol, to prevent hormone-mediated sexual receptivity in Experiment 1, is in disagreement with the findings of a previous report (Fernández-Guasti, et al., 1985a). In contrast to the present study, Fernández-Guasti and coworkers found that systemic injections of either $\alpha_1$- or ß-adrenergic receptor antagonists inhibit lordosis in estrogen/progesterone-treated female rats. Their results suggest that both of these adrenergic receptor subtypes are required for the facilitative action of NE on lordosis. In support of the results from Experiment 1, Davis and Kohl were also unable to block lordosis with systemic injections of phenoxybenzamine, however they did inhibit receptivity when estrogen/progesterone-treated female rats were injected with the $\alpha_2$ receptor agonist clonidine (Davis and Kohl, 1977). This effect was blocked by the $\alpha_2$ receptor antagonist yohimbine, suggesting that either the presynaptic $\alpha_2$ receptor

was functioning to inhibit the release of NE or perhaps a postsynaptic $\alpha_2$ receptor was involved. It is unclear why systemic studies are in disagreement with respect to the adrenergic receptors involved and their function. Considering the vast complexity of the systems and variabilities in procedures it may not be a direct enough approach to the question. A better method may be to deplete the source of NE and replace it with a selective agonist as was done in Experiment 3. Results from the study using this model gives strong support to the hypothesis that $\alpha_1$-adrenergic receptor stimulation is necessary for lordosis. It is certainly likely that other receptor subtypes are involved as well.

Although NE does appear to be involved in the control of hormone-mediated lordosis, the location of NE's action within the neural circuit is unclear. To address this question, a number of investigators have performed experiments applying adrenergic agonists and antagonists, as well as NE, directly into areas of the brain thought to be important for the control of lordosis. As with the systemic studies, the direct application of adrenergic compounds into specific brain regions have produced conflicting results. One of the first studies in which selective adrenergic receptor ligands were injected into lordosis relevant brain regions suggested that blockade of $\alpha_1$ receptors or stimulation of ß-receptors in the VMN or MPN facilitated lordosis in estrogen-treated female rats (Foreman and Moss, 1978). In addition, stimulation of $\alpha_1$

receptors or blockade of ß-receptors in either of these regions reduced receptivity in estrogen-treated females. These results indicate that $\alpha_1$ stimulation in the VMN or MPN inhibits lordosis while ß-receptor stimulation in either of these regions facilitates lordosis in female rats. In this same study, injections of NE into the area of the VMN or MPN was found to facilitate lordosis. Others have shown however, that NE injection into the area of the MPN inhibited lordosis in female rats primed with estrogen and progesterone (Caldwell and Clemens, 1986). In yet another study, a combined injection of $\alpha_2$- and ß-receptor agonists into the VMN of estrogen-treated female rats facilitated lordosis, while separately they did not (Fernández-Guasti, et al., 1985b). Also in this study, NE injection into the VMN facilitated lordosis in estrogen-treated rats. Etgen has recently shown that implants of the $\alpha_1$ antagonist prazosin into the VMN of estrogen/progesterone-treated female rats prior to progesterone treatment, prevents the females from becoming receptive (Etgen, 1990). This would indicate that $\alpha_1$-receptors in the VMN are necessary for lordosis. In Experiment 2 of the present study, NE injections into the VMN of estrogen-treated female rats failed to facilitate receptivity. It is thought that NE functions selectively in brain regions such as the VMN or MPN to effect lordosis. However, as seen with systemic studies, results from site specific studies are conflicting and inconsistent and do not

agree with regard to the role NE plays in the display of lordosis, specific to these brain regions.

Although NE is required for lordosis, its action may not be at a particular site but throughout various regions within the neural circuits controlling receptivity. Convincing evidence that NE is not required in a specific region important for sexual behavior is given by the results of Experiment 3. Depletion of NE in either the VMN or MPN did not reduce lordosis in ovariectomized, estrogen/progesterone-treated female rats. Furthermore, although i.c.v. injection of the $\alpha_1$-adrenergic receptor agonist phenylephrine restored lordosis in VNAB-lesioned females, selective injection of phenylephrine into either the VMN or MPN of VNAB-lesioned females failed to reinstate receptivity. These results strongly suggest that noradrenergic neurons in the VMN or MPN alone are not necessary for lordosis in ovariectomized, estrogen/progesterone-treated female rats. Although NE concentrations have been shown to increase in the VMN when estrogen/progesterone-treated female rats are receptive (Vathy and Etgen, 1989), it is quite possible that NE concentrations are elevated in other areas as well.

NE transmission may be required in various brain regions either simultaneously or in a coordinated manner to effect the general excitation of neurons within the hormone-sensitive circuit which controls receptivity. Without the influence of

NE, neurons may be unable to reach the necessary level of excitation.

In conclusion, although previous reports indicate that regions such as the VMN and MPN are important for the control of lordosis and NE is in some way required for this behavior, the results of the present study indicate that noradrenergic neurons terminating in either the VMN or MPN are not in and of themselves essential for gonadal steroid induction of sexual receptivity in ovariectomized female rats. Although $\alpha_1$-receptors stimulation most likely functions to increase the transmission resulting in lordosis it is probable that this is not specific to one brain region but is a generalized effect. Other adrenergic receptor subtypes are probably involved in the hormone-sensitive neural pathway for lordosis as well, but their specific involvement has not been addressed using the NE-depleted female rat model.

Further studies utilizing NE-depleted female rats could possibly clear up some of the discrepancies associated with the function of NE in the control of lordosis. By this method the female's endogenous source of NE is drastically reduced, and adrenergic stimulation replacement can be controlled as to locale and receptor subtype. It would be interesting to determine if a ß-adrenergic agonist could restore receptivity in VNAB-lesioned females as did the $\alpha_1$-adrenergic agonist. Neuropeptides thought to be involved in the control of lordosis could also be administered to VNAB-lesioned females

to investigate interactions between these neuromodulators and the noradrenergic system.

It is important to keep in mind the type of hormone-treatment models that are being used when comparing the results from different studies. The varying effects of steroid hormones and time frames of action are not yet understood and may greatly influence the effects of NE on the neurons controlling sexual receptivity. It would be interesting to investigate the temporal effects of NE on estrogen/progesterone induced receptivity. The reduced concentrations of NE at the time of hormone treatment could play a significant role in the modulation of lordosis.

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