ESTROGEN AND 5-HT_{1A} RECEPTOR FUNCTION

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Date

To the Associate Vice President for Research and Dean of the Graduate School:

I am submitting herewith a dissertation written by Astra E. Jackson entitled "ESTROGEN AND 5-HT$_{1A}$ RECEPTOR FUNCTION." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Molecular Biology.

Lynda Uphouse
Lynda Uphouse, Major Professor

We have read this dissertation and recommend its acceptance:

Department Chair

Accepted

Leslie M Thompson
Associate Vice President for Research and Dean of the Graduate School

DEDICATION

This study is dedicated to my mother, Helen Fisher Jackson, and to the memory of my father, Fred Norman Jackson.

ACKNOWLEDGEMENTS

It is with sincere appreciation that I acknowledge my mentor, Dr. Lynda Uphouse. Without her guidance and consistent support I would never have made it to the end of this journey; or, as she would say, to the beginning of the next journey. I thank her for the times she lead me, the times she pushed me, and for the times she carried me. May God continue to bless and keep her.

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Finally, to my mother, Helen Jackson, who is the best fan a girl could ever have. Her teachings have been the most important lessons I could expect to learn. Her prayers and encouragement have taken me far beyond the place I imagined I could reach.

ABSTRACT

ESTROGEN AND 5-HT_{1A} RECEPTOR FUNCTION

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The effects of estrogen treatment on 5-HT_{1A} receptor function were examined. When ovariectomized rats were given a single treatment with 25 μg estradiol benzoate followed 48 hr later with 500 μg progesterone, the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT), inhibited lordosis behavior. However, when rats were given a second estradiol benzoate injection (seven days later) followed by progesterone, the ability of 8-OH-DPAT to inhibit lordosis behavior was reduced. The reduction in the ability of 8-OH-DPAT to inhibit lordosis behavior lasted for at least 10 days, but after 15 days the inhibitory effects of 8-OH-DPAT on lordosis behavior had been restored. The protective effects of estrogen were also apparent when 17ß-estradiol was infused into the ventromedial nucleus of the hypothalamus (VMN) seven days before the second treatment with systemic estradiol benzoate. Consistent with hormonal changes that occur during the estrous cycle, estradiol benzoate also reduced the effectiveness of

8-OH-DPAT in inhibiting lordosis behavior 48 hr after treatment. Although the mechanisms for the protection of estrogen against the effects of 8-OH-DPAT on lordosis behavior are unknown, rats coinfused with N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (dibutyryl cAMP) and 8-OH-DPAT showed less inhibition of lordosis behavior than rats infused with 8-OH-DPAT alone. These data are consistent with the suggestion that estrogen activates a neural "cascade" that leads to a reduction in functioning of 5-HT_{1A} receptors.

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CHAPTER I

INTRODUCTION

The essential action of gonadal hormones in the elicitation of reproductive behavior by the female rat is well established. Reproductive behaviors include proceptive behaviors (which consist of hopping, darting, ear-wiggling and a rapid oscillatory head movement) and receptive behavior (lordosis reflex) (Pfaff et al., 1972; Beach, 1976). The lordosis reflex is a supraspinal reflex exhibited by the female in response to tactile stimulation by the male and is required for intromission during copulation (Pfaff and Modianos, 1985). Because destruction of the ventromedial nucleus of the hypothalamus (VMN) significantly reduces lordosis behavior (Matthews and Edwards, 1977), the VMN is assumed to be an essential component in the lordosis reflex circuitry (Pfaff and Sakuma, 1979). The VMN is also a site where gonadal hormones act to facilitate lordosis behavior (Rubin and Barfield, 1980). In ovariectomized rats, although replacement with estrogen alone can restore lordosis behavior (Powers, 1970), the behavior is accentuated by the addition of progesterone (Whalen, 1974; Fadem et al., 1979). For both hormones, localized application within the VMN is sufficient to elicit the behavior (Barfield and Chen, 1977; Rubin and Barfield, 1980;

Pleim and Barfield, 1988). Exactly how estrogen and/or progesterone modulate the VMN to increase lordosis behavior is not known.

In target cells, estrogen binds to intracellular receptors and thereby causes changes in the synthesis of mRNA and proteins (Cohen and Pfaff, 1981). Specific estrogen-induced changes in the number of terminals and contacts in ovariectomized, estrogen-primed rats have been described by Carrer and Aoki (1982). An increase in axodendritic synapses has also been shown (Nishizuka and Pfaff, 1989). Changes in protein synthesis appear to be required for lordosis behavior, since inhibition of protein synthesis within the VMN with anisomycin prevents estrogen's ability to enhance lordosis behavior (Meisel and Pfaff, 1985).

Estrogen modulates several neurotransmitters, including serotonin (5-HT) (Menard et al., 1992; Kow et al., 1994; McCarthy, 1995). 5-HT release (Vogel et al., 1970) and reuptake (Endersby and Wilson, 1973), as well as 5-HT transporter mRNA (McQueen et al., 1997) are all affected by estrogen. Additionally, serotonin receptors are modulated by estrogen (Biegon et al., 1982; Biegon and McEwen, 1982). Early evidence suggested that estrogen decreased the density of 5-HT_1 binding sites in brain tissue including the hypothalamus (Biegon and McEwen, 1982), but in more recent studies no such change was detected after estrogen treatment (Frankfurt et al., 1994). Clarke and Maayani (1990) found chronic estrogen treatment had no effect on the density of 5-HT_{1A} receptors in hippocampal membranes. Modulation by

estrogen of 5-HT receptors may depend on the brain area studied and/or the length of hormone treatment. In addition, various 5-HT receptors may undergo differential regulation. For example, 5-HT_2 receptor density has been reported to be increased by chronic estrogen treatment (Beigon et al., 1983). Recently, Sumner and Fink (1993) reported that estradiol stimulated an increase in the amount of 5-HT_{2A} receptor mRNA in the dorsal raphe nucleus of the female rat and, later, they found that the density of 5-HT_{2A} receptors, as measured by in vitro binding of [^{3}H]ketanserin, was significantly increased in female rat forebrain (Sumner and Fink, 1995). Moreover, the density of 5-HT_{2A} receptors in forebrain was higher in proestrous rats than in diestrous rats (Sumner and Fink, 1997).

The 5-HT receptor family is divided into 7 classes, based on amino acid sequence, pharmacological profile and signal transduction mechanism. The largest class to date, the 5-HT_1 class, includes five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}). Among the various 5-HT receptors that influence lordosis behavior, much attention has been focused on the 5-HT_{1A} receptor (Ahlenius et al., 1986; Uphouse et al., 1991a). A member of the G-protein coupled receptor family, the 5-HT_{1A} receptor negatively regulates adenylyl cyclase (DeVivo and Maayani, 1985; Yocca and Maayani, 1990; Zifa and Fillion, 1992). 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase has been demonstrated in

the rat and guinea pig hippocampus (DeVivo and Maayani, 1985). Bockaert et al. (1987) described similar results with forskolin-stimulated and vasointestinal polypeptide-stimulated adenylyl cyclase in the mouse and guinea pig hippocampus. Additionally, Andrade et al. (1986) reported that 5-HT_{1A} receptors may be coupled to K^+ channels. It is generally agreed that activation of 5-HT_{1A} receptors results in a decrease in the probability of neuronal firing. Activation of 5-HT_{1A} receptors, specifically those in the VMN, inhibits lordosis behavior (Ahlenius et al., 1986; Mendelson and Gorzalka, 1986; Aiello-Zaldivar et al., 1992; Uphouse et al., 1992).

Since activation of VMN 5-HT_{1A} receptors is incompatible with lordosis behavior, it is reasonable to assume that as the female moves from the sexually non-receptive state (diestrus) to the sexually receptive state (proestrus), the activation of 5-HT_{1A} receptors or their functional impact must be diminished. The overall effect of 5-HT_{1A} receptors in the VMN might be diminished in a number of ways including: (1) a decrease in endogenous release of 5-HT; (2) a decrease in the density of 5-HT_{1A} receptors; or (3) an altered cellular response following activation of 5-HT_{1A} receptors. Evidence to date leads us to hypothesize that a reduced cellular response is the most likely explanation. For example, Sumner and Fink (1993), using in situ hybridization, found no effect of estradiol on 5-HT_{1A} receptor mRNA in the VMN. Although 5-HT_{1A} receptor density in the VMN was unchanged

following estrogen treatment (Frankfurt et al., 1994), estrogen treatment alters the impact of activation of 5-HT_{1A} receptors in the VMN (Uphouse et al., 1994; Jackson and Uphouse, 1996).

Inhibition of lordosis behavior following infusion of the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT), into the VMN is attenuated by estrogen priming (Uphouse et al., 1994; Jackson and Uphouse, 1996). When ovariectomized rats were treated a single time with 25 μg estradiol benzoate, followed 48 hr later with 500 μg progesterone, inhibition of lordosis behavior was seen after intracranial infusion with doses of 8-OH-DPAT as low as 200 ng. However, after a second week of hormone priming, the dose response curve for 8-OH-DPAT was shifted to the right (Jackson and Uphouse, 1996; Trevino et al., 1998). It is assumed that the first treatment with estrogen initiated a cascade of neurochemical events which ultimately reduced the effectiveness of the 5-HT_{1A} receptor agonist. Estrogen's protective effect required 3 to 4 days to develop and lasted for at least 7 days from the initial estrogen treatment (Jackson and Uphouse, 1996). The duration of the protective effects of estrogen is addressed in the first of the experiments reported herein.

The results reported by Jackson and Uphouse (1996) are consistent with the suggestion that estrogen reduces 5-HT_{1A} receptor function in the VMN; however, the area of the brain in which the action of estrogen occurs is unknown. In the present report, estrogen was applied to the VMN to

determine if localized application was sufficient to attenuate the effects of 8-OH-DPAT. Additionally, the question of whether estrogen reduces the effectiveness of 5-HT_{1A} receptors on a time scale that is consistent with hormonal changes that occur during the estrous cycle was addressed.

Finally, the possibility that estrogen attenuates the effects of 8-OH-DPAT by increasing cAMP was examined. 5-HT_{1A} receptors are negatively coupled to adenylyl cyclase and Kow et al. (1994) have reviewed evidence that allows the suggestion that lordosis behavior would be reduced by neurotransmitters that inhibit adenylyl cyclase and facilitated by neurotransmitters that increase adenylyl cyclase. For example, activation of dopaminergic D_2 receptors and 5-HT_{1A} receptors has been shown to inhibit lordosis behavior (Grierson, et al., 1988; Mendelson, 1992). Both have also been shown to be negatively coupled to the adenylyl cyclase pathway (Kebabian, et al., 1972; DeCamilli, et al., 1979; Yocca and Maayani, 1990; Hoyer and Schoeffter, 1991). Consistent with this idea was the finding that a water-soluble derivative of forskolin protected against the lordosis-inhibiting effects of 8-OH-DPAT in proestrous rats (Uphouse et al., 1997). Because estrogen increases cAMP in the mediobasal hypothalamus (Kow et al., 1994), it is possible that estrogen's protection against the effects of 8-OH-DPAT on lordosis behavior results from this increase in cAMP. If so, then a compound such as dibutyryl cAMP, that mimics the effects of cAMP, should also mimic

estrogen's protection against the inhibitory effects of 8-OH-DPAT on lordosis behavior.

The following specific questions were addressed:

1. Does prior treatment with estrogen, infused directly into the ventromedial nucleus of the hypothalamus, protect against the lordosis-inhibiting effects of 8-OH-DPAT?

2. Can estrogen dose-dependently reduce the ability of 8-OH-DPAT to inhibit lordosis behavior on a time scale comparable to that required to elicit lordosis behavior?

3. Does dibutyryl cAMP infused into the ventromedial nucleus of the hypothalamus mimic estrogen's ability to alter the effect of 8-OH-DPAT on lordosis behavior?

CHAPTER II

MATERIALS AND METHODS

Materials:

Fischer (CDF-344) female rats were purchased from Sasco Laboratories, Wilmington, MA. Hormones were purchased from Fisher Scientific, Houston, TX. The 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT), was purchased from Research Biochemicals, Inc., Natick, MA. N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (dibutyryl cAMP) was purchased from Sigma Chemical Company, St. Louis, MO.

Methods:

Experiment 1:

Duration of estradiol benzoate action on the effects of 8-OH-DPAT.

Female, Fischer-344 rats, weaned at 25 days of age, were housed 2 or 3 per cage with like-sex littermates in a colony room with a 12L-12D cycle (lights on at 12 midnight) and ad lib access to food (rat chow) and water. Rats were bilaterally ovariectomized at 80-100 days of age. Fourteen days after ovariectomy, rats were injected subcutaneously (s.c.) with 25 μg estradiol benzoate. A second injection with 25 μg estradiol benzoate was given

7 (n = 8), 10 (n = 9), 12 (n = 8), 15 (n = 8), or 17 (n = 9) days after the first injection; 48 hr later, rats were injected with 500 μg progesterone (s.c.). A control group of rats (n = 13) received a single injection with estradiol benzoate followed 48 hr later with progesterone. Estradiol benzoate was dissolved in sesame seed oil and progesterone was dissolved in propylene glycol. Hormones were administered in a volume of 0.1 ml/rat.

Four to six hr after progesterone, rats were injected intraperitoneally (i.p.) with 0.15 mg/kg 8-OH-DPAT dissolved in 0.9% saline. 8-OH-DPAT was injected in a volume of 1.0 ml/kg body weight. Prior to injection with 8-OH-DPAT, the female was placed in the home cage of a sexually experienced male and her sexual behavior was recorded for a minimum of 10 mounts. The female was then removed, injected with 8-OH-DPAT, and returned to the male's cage. Lordosis behavior was recorded consecutively for the next 30 minutes. Lordosis to mount ratios (L/M) were computed for the pretest and for each of six 5-min intervals after injection. At the conclusion of the experiment, ovariectomy was confirmed by postmortem examination.

Data were evaluated by two-way, repeated measures ANOVA with days between estradiol benzoate treatments as the main factor and time after 8-OH-DPAT as the repeated factor. Differences between doses of estradiol benzoate (within time after 8-OH-DPAT) were evaluated by the Tukey test. Time-dependent effects (within treatment condition) were evaluated by

Dunnett's test. The statistical reference was Zar (1996) and an alpha level of 0.05 was required for rejection of the null hypothesis.

Experiment 2:

The ventromedial nucleus of the hypothalamus as the site of estrogen action.

The following experiment was designed to test the hypothesis that estrogen, infused directly into the VMN, during the first week of hormone treatment would protect against the lordosis-inhibiting effects of 8-OH-DPAT in a second week of hormone treatment. Female, Fischer-344 rats, 80-100 days old, were implanted bilaterally with 22-gauge stainless-steel guide cannulae advanced stereotactically into the VMN [atlas coordinates from Konig and Klippel (1963) AP 4.38; DV 7.8; ML 0.4]. Rats were bilaterally ovariectomized two weeks after the implant surgery. Five to eight days after ovariectomy, females were divided into four experimental groups. GROUP 1 received a 3 ng 17ß-estradiol (dissolved in propylene glycol) infusion into the VMN at a rate of 0.24-0.26 µl/min to a final volume of 0.5 µl/bilateral site. Seven days later rats were injected subcutaneously (s.c.) with 25 µg estradiol benzoate. Forty-eight hr later, 500 µg progesterone were injected (s.c.). GROUP 2 received propylene glycol (0.24-0.26 µl/min to a final volume of 0.5 µl/bilateral site) into the VMN. Seven days later, group 2 rats were injected with 25 µg estradiol benzoate (s.c.) followed with 500 µg progesterone forty-eight hr later. GROUP 3 received only one 25 µg estradiol benzoate injection (s.c.) followed 48 hr later with 500 µg progesterone (s.c.). GROUP 4 received

a 25 μg estradiol benzoate injection (s.c.) plus infusion of propylene glycol into the VMN at a rate of 0.24-0.26 μl/min to a final volume of 0.5 μl/bilateral site.

In all groups, sexual behavior testing began 3-5 hr after progesterone injections. Testing for sexual behavior, as previously described by Uphouse et al. (1991b), was initiated within the first 1-3 hours after lights off (lights off 12:00 noon to 12:00 midnight, CST). The females were placed with a sexually-experienced male within a CMA/120 containment system (BAS) as previously described by Uphouse et al. (1994). Sexual behavior was monitored prior to and during the infusion of 8-OH-DPAT and for 30 consecutive min after the infusion. Sexual receptivity was quantified as the lordosis to mount ratio (L/M) (e.g. number of lordosis responses by the female divided by the number of mounts by the male). For statistical purposes, the data were grouped into the pretest period, infusion period, and six consecutive 5 min intervals after infusion.

At the conclusion of the experiment, females were anesthetized with Methoxyflurane (Metofane) and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brain was excised and placed in 10% buffered formalin for a minimum of 24 hr before vibratome sectioning (100 μm). Tissue sections were stained with cresyl violet and cannulae locations were verified according to Konig and Klippel (1963). The location of each cannula was determined by an individual without knowledge of the

experimental treatment or behavioral results. Cannula location was indicated as the first section in which the most ventral location of the cannula occurred. Only data from rats with both cannulae located within the VMN were included in the study. Ovariectomy was confirmed by postmortem examination. Data were evaluated by two-way, repeated measures ANOVA with type of hormone treatment as the independent factor and time after 8-OH-DPAT as the repeated factor. The statistical reference was Zar (1996) and an alpha level of 0.05 was required for rejection of the null hypothesis.

Experiment 3:

Dose-dependent effects of estrogen on the response to 8-OH-DPAT.

The following study was performed to determine whether dose-dependent effects of estradiol benzoate on 8-OH-DPAT's ability to inhibit lordosis behavior were present on a time scale comparable to that required for estrogen to elicit lordosis behavior. Rats were bilaterally implanted and ovariectomized as described in Experiment 2. Seven to ten days after ovariectomy, rats were injected with 2.5, 7.5 or 25 μg estradiol benzoate (s.c.) followed 48 hr later with progesterone (500 μg). Three to five hr after progesterone injections, evaluation of sexual receptivity and testing of sexual behavior, as described in Experiment 2, began. Sexually-receptive females were infused with 50, 100, or 200 ng 8-OH-DPAT. Sexual behavior was recorded prior to, during, and for 30 consecutive minutes after 8-OH-DPAT infusion. Data were evaluated by repeated measures ANOVA (with time after

infusion as the repeated factor) and with dose of estradiol benzoate and dose of 8-OH-DPAT as the independent factors. Cannulae locations and ovariectomy were verified as described in Experiment 2.

Experiment 4:

Effects of dibutyryl cAMP on the lordosis-inhibiting effects of 8-OH-DPAT.

The following experiment was performed to determine if a compound which would mimic an increase in cAMP would mimic estrogen's ability to reduce the effect of 8-OH-DPAT to inhibit lordosis behavior. Rats were bilaterally implanted and ovariectomized as previously described. Rats (EP) were hormone primed with 25 μg estradiol benzoate (s.c.) followed 48 hr later by 500 μg progesterone. Rats were infused with either 200 ng 8-OH-DPAT or with 200 ng 8-OH-DPAT and 50 μg N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (dibutyryl cAMP dissolved in H_2O) into the VMN (0.5 μl/site; 0.24-0.26 μl/min). An additional group (EEP) received two 25 μg estradiol benzoate injections (separated by seven days). Forty-eight hr after the second estradiol benzoate injection, these rats were injected with 500 μg progesterone (s.c.). Four to six hr later, rats were infused with 200 ng 8-OH-DPAT. During the infusion of the drugs into the VMN, rats were gently hand-restrained while the infusion was controlled by a CMA/100 microinjector. Sexual behavior was recorded in all groups prior to, during, and for 25 consecutive minutes after the 8-OH-DPAT infusion or 8-OH-DPAT and dibutyryl cAMP coinfusion. Data were evaluated by repeated measures

ANOVA with time after the 8-OH-DPAT infusion as the repeated factor and hormone treatment and drug treatment as the independent factors.

CHAPTER III

RESULTS

Experiment 1:

Duration of estradiol benzoate action on the effects of 8-OH-DPAT.

In previous studies, we have shown that ovariectomized rats, primed with a single treatment of 25 μg estradiol benzoate followed 48 hr later with 500 μg progesterone, show a robust decline in lordosis behavior when treated with 8-OH-DPAT. Such inhibition of lordosis behavior following 8-OH-DPAT does not occur, however, when rats are primed with two estradiol benzoate injections that are separated by 3-7 days. The following experiment was designed to determine the duration of this protective action of estrogen against the inhibitory effect of 8-OH-DPAT on lordosis behavior.

When rats received a single injection with estradiol benzoate followed 48 hr later by progesterone, an i.p. treatment with 0.15 mg/kg 8-OH-DPAT significantly inhibited lordosis behavior by 5 min after injection, and the inhibition continued throughout the 30 min test period (Dunnett's, all $q_{294, 6} \geq 2.51$, $p \leq 0.05$) (Figure 1). The effect of 8-OH-DPAT was attenuated when a second injection with estradiol benzoate occurred 7 or 10 days after the first

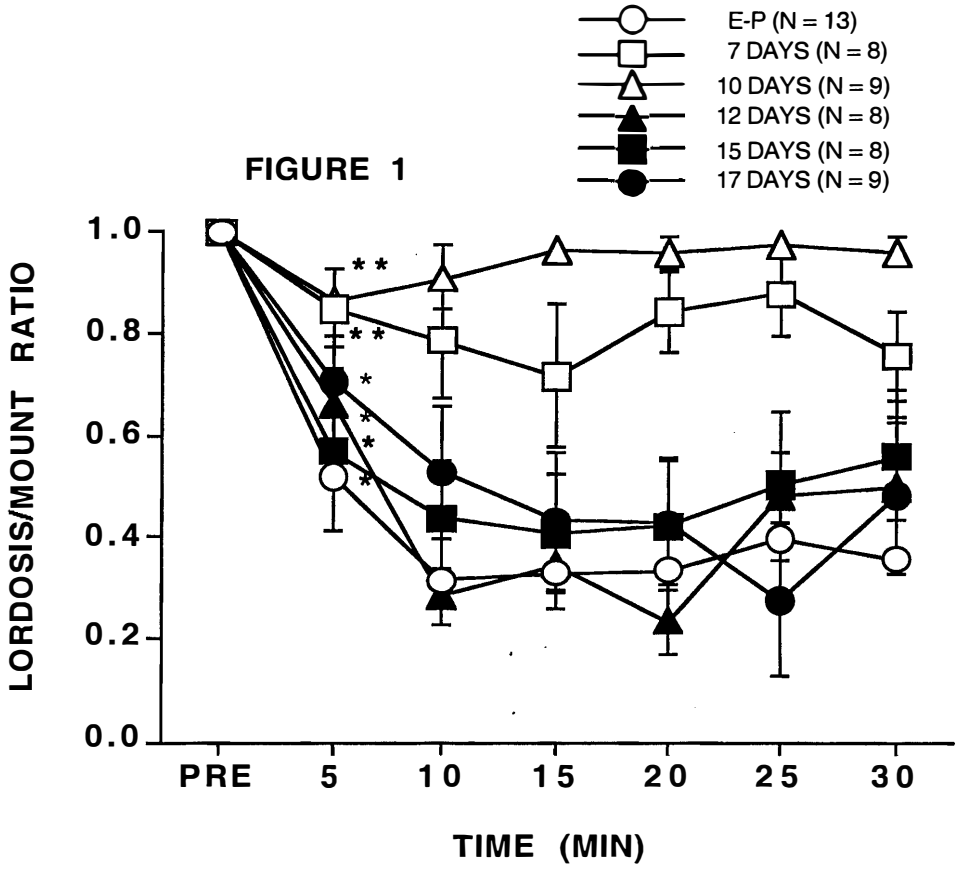

Figure 1: Duration of estrogen's protection against the lordosis-inhibiting effects of 8-OH-DPAT.

Rats were injected (s.c.) with 25 μg estradiol benzoate on the day of ovariectomy and received a second estradiol benzoate injection 7 (7 DAYS), 10 (10 DAYS), 12 (12 DAYS), 15 (15 DAYS) or 17 (17 DAYS) days later. N's respectively are 8, 9, 8, 8 and 9. Forty-eight hr after the second estradiol benzoate treatment, rats were injected s.c. with 500 μg progesterone. Behavioral testing took place 4 to 6 hr after progesterone. Data are the mean ± S.E. L/M ratios before injection (PRE) and for each 5 min interval after injection with 0.15 mg/kg 8-OH-DPAT (i.p.). Also shown are the mean L/M ratios following treatment with 8-OH-DPAT for 13 rats (EP) given a single injection of 25 μg estradiol benzoate on the day of ovariectomy followed 48 hr later with progesterone. Single asterisks indicate the first 5 min interval during which L/M ratios were significantly different from the pretest. Double asterisks indicate the first 5 min interval where differences were present between rats given 2 treatments with estradiol benzoate and rats treated only once (EP) with the hormone.

hormone treatment. For these rats, the lordosis/mount ratio was never significantly different from the pretest (Dunnett's, all $p > 0.05$). However, when the two treatments with estradiol benzoate were separated by 12, 15, or 17 days, 8-OH-DPAT's effect was comparable to that of rats treated a single time with estradiol benzoate. Lordosis/mount ratios of rats given two estradiol benzoate treatments, separated by 12 or more days, were significantly lower than the pretest score throughout the 30 min testing period (Dunnett's, $q_{294, 6} \geq 2.51$, $p \leq 0.05$). There were significant effects of number of days between treatments with estradiol benzoate ($F_{5, 49} = 9.54$, $p \leq 0.0001$), of time after injection with 8-OH-DPAT ($F_{6, 294} = 31.54$, $p \leq .0001$), and their interaction ($F_{30, 294} = 2.64$, $p \leq 0.0001$). These findings are consistent with previous results suggesting estradiol benzoate reduces the effects of 8-OH-DPAT. Where estrogen acts in the brain was investigated in the following experiment.

Experiment 2:

The ventromedial nucleus of the hypothalamus as the site of estrogen action.

A. Preliminary studies

In preliminary studies, two doses of 17ß-estradiol (1.5 ng/0.5 µl and 3 ng/0.5 µl) were infused into the VMN. The objective of this preliminary investigation was to determine if these doses of 17ß-estradiol would fail to produce vaginal cornification following infusion into the VMN. As seen in

Table I, eleven rats received bilateral infusion of 1.5 ng 17ß-estradiol into the VMN and four rats received 3 ng 17ß-estradiol bilaterally into the VMN. Forty-eight hr later, none of these rats showed cornification of vaginal cells. All rats had vaginal smears typical of an ovariectomized rat with no hormone priming (eg. smears containing cellular debris with little or no epithelial cells). Additionally, some females were checked for display of lordosis behavior by placing them in the home cage of a sexually experienced male. Six rats that received 1.5 ng 17ß-estradiol were tested for lordosis behavior and 2 showed some lordosis behavior. All four of the rats that received 3 ng 17ß-estradiol were tested; however, none displayed lordosis behavior. In week 2 (seven days after the infusion of 17ß-estradiol), all rats were injected with 25 µg estradiol benzoate s.c. followed 48 hr later with 500 µg progesterone s.c. Both groups of rats were again checked for lordosis behavior. In week 2, in the group that previously received 1.5 ng 17ß-estradiol, 8 of 11 showed lordosis behavior above a 0.75 lordosis/mount ratio and were considered to be sexually receptive. Of the 4 rats that received 3 ng 17ß-estradiol, all were sexually receptive in week 2. Sexually-receptive rats in each group were tested after treatment with 8-OH-DPAT. Of the 8 receptive rats previously infused with 1.5 ng 17ß-estradiol, one was not inhibited following injection with 8-OH-DPAT. Of the remaining 7, 4 received 0.15 mg/kg 8-OH-DPAT (i.p.) and 3 received 200 ng 8-OH-DPAT (i.c.). All 7 rats were inhibited by 8-OH-DPAT. All receptive rats, previously treated i.c. with 3 ng 17ß-estradiol, were

Table 1.

Data from preliminary experiments with 17ß-estradiol, i.c.

Dose of 17ß-estradiol	number of animals	vaginal cornification at 48 hr	number tested for sexual receptivity week 1	number showing lordosis behavior week 1	number tested for sexual receptivity week 2	number with l/m ratio > 0.75 in week 2	number inhibited with 8-OH-DPAT*
1.5 ng	11	0/11 (0%)	6	2/6 (33%)	11	8/11 (73%)	7/8 (88%)
3 ng	4	0/4 (0%)	4	0/4 (0%)	4	4/4 (100%)	2/4 (50%)

* Inhibition was defined as lordosis/mount ratio < 0.75 for two consecutive 5 min intervals

Shown are the effects of infusion of 17ß-estradiol into the ventromedial nucleus of the hypothalamus. Data are for 11 rats infused bilaterally with 1.5 ng 17ß-estradiol and for 4 rats infused with 3 ng 17ß-estradiol in week 1 and injected s.c. with 25 µg estradiol benzoate and 500 µg progesterone in week 2. Shown are the number of rats infused bilaterally with 1.5 ng or 3 ng 17ß-estradiol and the numbers of animals showing vaginal cornification 48 hr after infusion. Also given are the numbers in each group that were tested for sexual receptivity and the number showing lordosis behavior in week 1. Shown are the number showing sexual receptivity in week 2 and the effects of 8-OH-DPAT.

treated with 200 ng 8-OH-DPAT i.c. Two of the 4 rats were inhibited by 200 ng 8-OH-DPAT i.c. The lordosis/mount ratios for rats given 8-OH-DPAT i.c. are shown in Figure 2. On the basis of these preliminary studies, 3 ng 17ß-estradiol was chosen to more fully evaluate the hypothesis that estrogen, infused into the VMN, can protect against the lordosis-inhibiting effects of 8-OH-DPAT.

B. Effects of prior VMN infusion with 3 ng 17ß-estradiol.

To test the hypothesis that prior treatment with estrogen into the VMN would protect against the lordosis-inhibiting effects of 8-OH-DPAT, ten rats were preinfused with 3 ng 17ß-estradiol. None of these rats had cornified vaginal cells 48 hr after infusion with 17ß-estradiol (Table 2). Therefore, it is reasonable to assume that the hormone did not reach high levels in peripheral tissues. In week two, after s.c. priming with estradiol benzoate and progesterone, all 10 of these rats showed high levels of sexual receptivity. Inhibition of lordosis behavior by 8-OH-DPAT was observed in only 5 of the rats.

Eight rats were preinfused with propylene glycol (Table 2). Forty-eight hr later, none of the rats had cornified vaginal cells. All eight rats showed high sexual receptivity in week 2 and lordosis behavior was inhibited in 7 of the 8 rats tested with 8-OH-DPAT. A third group of rats received estradiol benzoate s.c. without any prior treatment. Vaginal smears were taken forty-eight hr later. All rats in the third group showed vaginal cornification

FIGURE 2

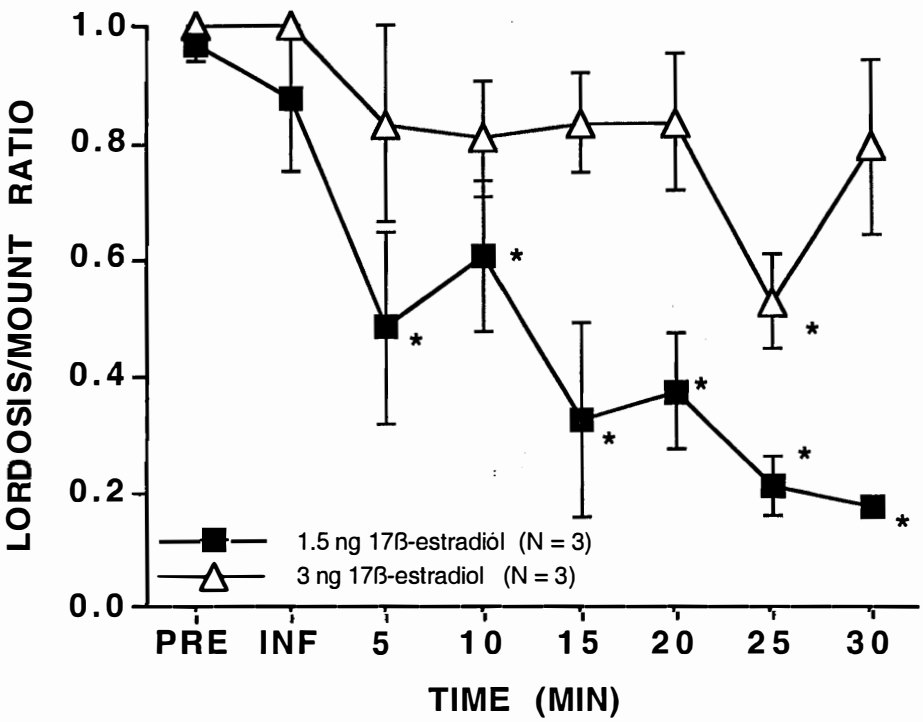

Figure 2: Preliminary studies with 17ß-estradiol infusion into the VMN. Shown are the mean ± S.E. L/M ratios for rats pretreated with 17ß-estradiol (1.5 ng or 3 ng) into the VMN and treated one week later with 25 μg estradiol benzoate s.c. and 500 μg progesterone. N's are 3 and 3, respectively. Data are the mean ± S.E. L/M ratios prior to (PRE), during (INF), and for 30 consecutive min following VMN infusion with 200 ng 8-OH-DPAT. Single asterisks indicate a significant difference from the pretest interval.

Table 2.

Results of 17ß-estradiol into the VMN.

Pretreatment	Treatment 2nd week	number of animals	week 1 vaginal cornification at 48 hr after first treatment	week 2 vaginal cornification at 48 hr after first treatment	number with l/m ratio > 0.75 week 2	number inhibited by 8-OH-DPAT*
3 ng 17ß-estradiol (in propylene glycol)	25 µg estradiol benzoate s.c. (in sesame seed oil)	10	0/10 (0%)	10/10 (100%)	10/10 (100%)	5/10 (50%)
propylene glycol	25 µg estradiol benzoate s.c. (in sesame seed oil)	8	0/8 (0%)	8/8 (100%)	8/8 (100%)	7/8 (88%)
none	25 µg estradiol benzoate s.c. (in sesame seed oil)	6	—	6/6 (100%)	5/6 (83%)	5/5 (100%)
none	25 µg estradiol benzoate s.c. (in sesame seed oil) with PG in the VMN	5	—	5/5 (100%)	4/4 (100%)	4/4 (100%)

* Inhibition was defined as lordosis/mount ratio < 0.75 for two consecutive 5 min intervals

Table 2

The effects of various pretreatment conditions and treatment conditions in week 2 are shown in Table 2. Data are for 10 rats infused with 3 ng 17ß-estradiol in week 1 and injected s.c. with 25 μg estradiol benzoate and 500 μg progesterone in week 2 as described in the Methods. Also shown are data for 8 rats infused with 0.5 μl propylene glycol in week 1 and injected s.c. with 25 μg estradiol benzoate and progesterone in week 2. Data are also shown for 6 rats that received an injection of 25 μg estradiol benzoate s.c. and 500 μg progesterone in week 2. A final group of five rats was infused with 0.5 μl propylene glycol and also injected with 25 μg estradiol benzoate s.c. and 500 μg progesterone in week 2.

indicative of estrogen priming but only five showed sexual receptivity sufficient for testing of 8-OH-DPAT. All five rats showed inhibition of lordosis behavior following infusion with 8-OH-DPAT (Table 2).

A final group of 5 rats received no prior treatment but received estradiol benzoate (s.c.) plus a 0.5 µl bilateral propylene glycol infusion into the VMN. Similar to the s.c. estradiol benzoate group, all five showed vaginal cornification 48 hr later. Four of the five showed high sexual receptivity and were infused with 8-OH-DPAT. Inhibition of lordosis behavior was seen in all four of these rats (Table 2).

Sites of the 3 ng 17ß-estradiol infusions are shown in Figure 3. Cannulae sites were clustered within the VMN as intended. No animal had cannulae sites outside the VMN and no cannula resided within the third ventricle.

There was a significant effect of type of treatment on lordosis/mount ratios ($F_{3, 23} = 4.64$, $p \leq 0.05$) with the inhibitory effect of 8-OH-DPAT attenuated by 3 ng 17ß-estradiol into the VMN (Figure 4A). There was also a significant time x drug effect ($F_{21, 161} = 2.01$, $p \leq 0.05$). Within 15 min of the 8-OH-DPAT infusion, there was some decline in the lordosis/mount ratios of all hormone treatment groups relative to their pretest ratios (Dunnett's, all $q_{161, 7} \geq 2.57$, $p \leq 0.05$). With the exception of rats previously infused with 3 ng 17ß-estradiol, lordosis/mount ratios of all groups remained suppressed throughout the duration of the 30-min testing period (Dunnett's, all $q_{161, 7}$

FIGURE 3

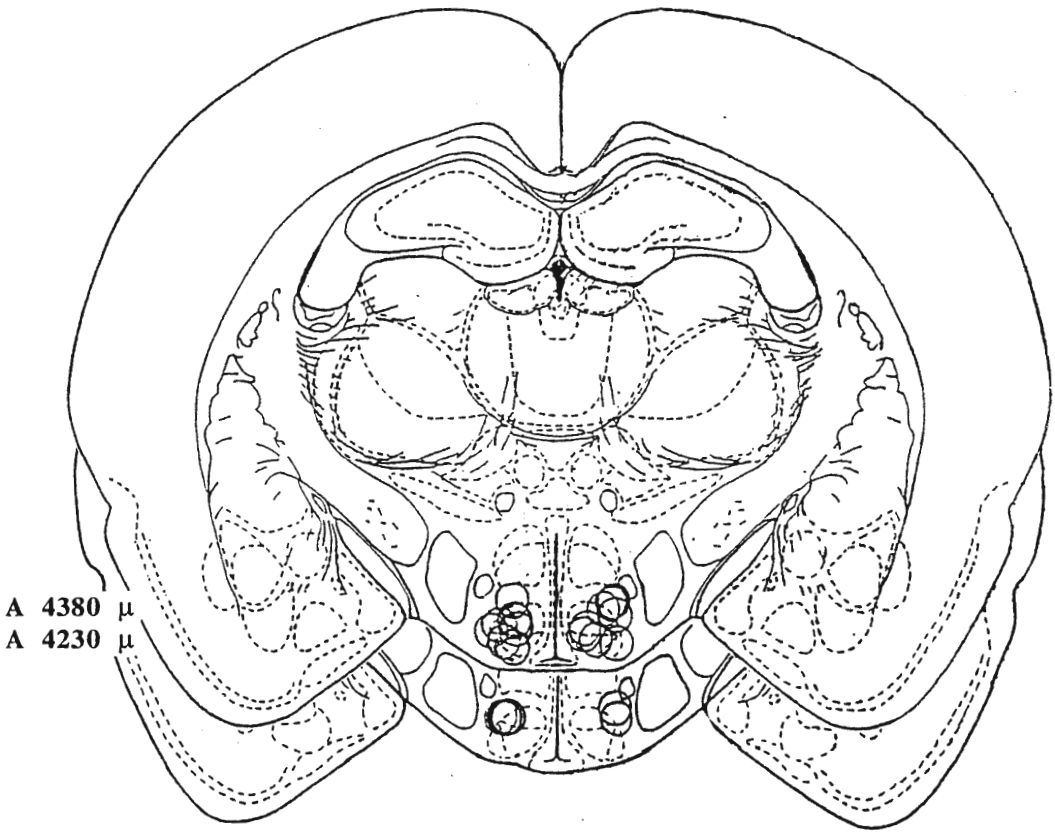

Figure 3: Infusion sites of 3 ng 17ß-estradiol into the VMN.

Coronal sections through the medial basal hypothalamus are shown in Figure 3. Shown are cannulae locations according to the atlas by Konig and Klippel (1963) for rats infused i.c. with 3 ng. Anterior/posterior locations are according to the atlas by Konig and Klippel (1963). Open circles (o) indicate sites where the most ventral location of cannulae were identified following histological evaluation as detailed in the Methods.

FIGURE 4

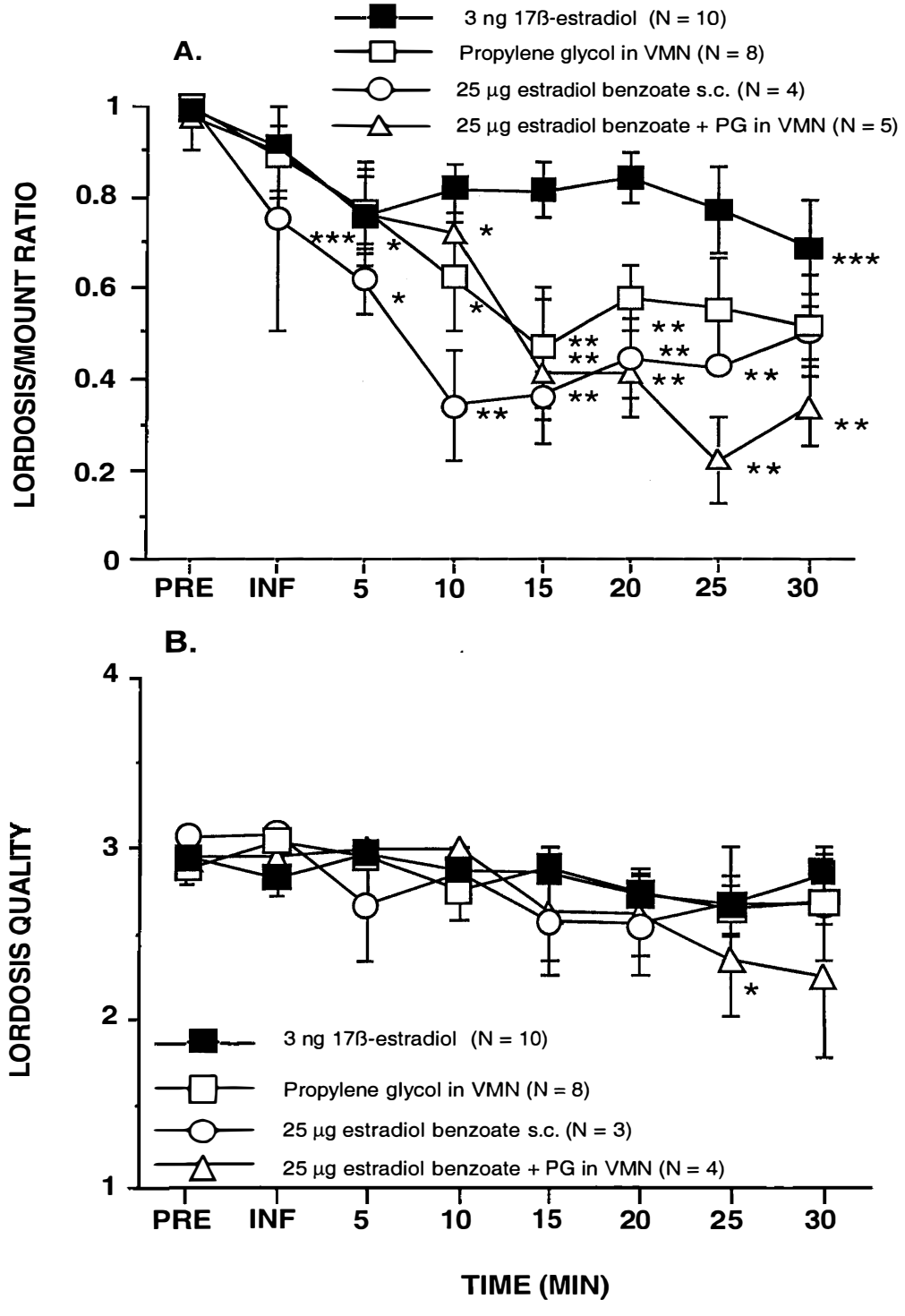
A.
3 ng 17ß-estradiol (N = 10)
Propylene glycol in VMN (N = 8)
25 µg estradiol benzoate s.c. (N = 4)
25 µg estradiol benzoate + PG in VMN (N = 5)
LORDOSIS/MOUNT RATIO
1
0.8
0.6
0.4
0.2
0
PRE
INF
5
10
15
20
25
30
B.
LORDOSIS QUALITY
4
3
2
1
3 ng 17ß-estradiol (N = 10)
Propylene glycol in VMN (N = 8)
25 µg estradiol benzoate s.c. (N = 3)
25 µg estradiol benzoate + PG in VMN (N = 4)
TIME (MIN)

Figure 4: Effect of hormone treatment on lordosis behavior and lordosis quality.

Shown in Figure 4A are the mean ± S.E. L/M ratios for rats pretreated with 3 ng 17ß-estradiol or propylene glycol into the VMN and treated one week later with 25 μg estradiol benzoate and 500 μg progesterone. N's are 10 and 8 respectively. Also shown are the mean ± S.E. L/M ratios for rats treated in the second week, only, with 25 μg estradiol benzoate and 500 μg progesterone or 25 μg estradiol benzoate (s.c.) and 0.5 μl propylene glycol (i.c.) into the VMN and then given 500 μg progesterone (n's 4 and 5 respectively). Data are the L/M ratios prior to (PRE), during (INF), and for 30 consecutive min following infusion with 200 ng 8-OH-DPAT. Single asterisks indicate the first 5 min interval during which there was a significant difference from the pretest interval. Double asterisks indicate a significant difference from the 3 ng treatment group within the same time interval. Triple asterisks indicate the intervals during which there was a significant difference from the pretest interval in the 3 ng treatment group. Mean lordosis quality scores are shown in Figure 4B. Single asterisks indicate significant differences from the pretest interval.

≥ 2.57, $p \leq 0.05$). In rats preinfused with 3 ng 17ß-estradiol, the lordosis/mount ratio was significantly different from the pretest interval only at 5 and 30 min, and the magnitude of inhibition was less than that ultimately seen in other groups. Rats that were preinfused with 3 ng 17ß-estradiol were significantly different from all other groups for a majority of the test intervals after infusion (Tukey's, $q_{161,4} \geq 3.63$, $p \leq 0.05$).

There was also a modest, but statistically significant, decline in lordosis quality after treatment with 8-OH-DPAT ($F_{7,147} = 5.72$ $p \leq .0001$) (Figure 4B). However, there was no significant treatment x time interaction for lordosis quality ($F_{21,147} = 1.18$ $p > 0.05$); with posthoc comparisons, only the estradiol benzoate plus propylene glycol group showed a significant decline in quality relative to its pretest value, and this occurred very late into the testing period.

In summary, a protective effect of estrogen against the lordosis-inhibiting effects of 8-OH-DPAT was seen when the first estrogen treatment was restricted to the CNS, within the vicinity of the VMN. The protection was comparable to that seen following prior systemic treatment with the hormone (Compare Figure 1 with Figure 4A).

Experiment 3:

Dose-dependent effects of estrogen on the response to 8-OH-DPAT.

The next experiment was designed to test the hypothesis that estrogen, on a time scale comparable to that required to elicit lordosis behavior, could dose-dependently reduce the ability of 8-OH-DPAT to inhibit lordosis

behavior. Rats were primed with 2.5, 7.5, or 25 μg estradiol benzoate followed 48 hr later with 500 μg progesterone. Four to six hr later, rats were tested for sexual behavior after VMN infusion with 50, 100, or 200 ng 8-OH-DPAT. The effect of these treatments are shown in Figure 5. In all groups, every dose of the 5-HT_{1A} receptor agonist significantly reduced the lordosis/mount ratio relative to the pretest period (ANOVA for time after infusion, $F_{6, 330} = 44.05$, $p \leq 0.0001$). Consequently, the overall effect of hormone treatment narrowly missed statistical significance ($F_{2, 55} = 2.89$, $p \leq .06$). Regardless of the hormone priming or dose of 8-OH-DPAT infused, the lordosis/mount ratio was significantly reduced by at least 10 min following infusion and remained inhibited through the remainder of the testing period (Dunnett's, $q_{6, 330} \geq 2.51$, $p \leq 0.05$). Consequently, there were no significant interactions between dose of estradiol benzoate or 8-OH-DPAT and time after infusion ($p > 0.05$).

With the exception of rats primed with the 2.5 μg dose of estradiol benzoate, there was little dose-dependency for 8-OH-DPAT over the range of 50 ng to 200 ng/bilateral site. For the 2.5 μg estradiol benzoate group, rats infused with 50 ng 8-OH-DPAT were significantly different from those infused with 200 ng 8-OH-DPAT at 15, 20 and 30 min after the infusion (Tukey's, $q_{330, 6} \geq 3.31$, $p \leq 0.05$). When the different estradiol benzoate treatments were examined within dose of 8-OH-DPAT, there were no significant differences in the effect of 50 or 100 ng 8-OH-DPAT across the three hormone

FIGURE 5

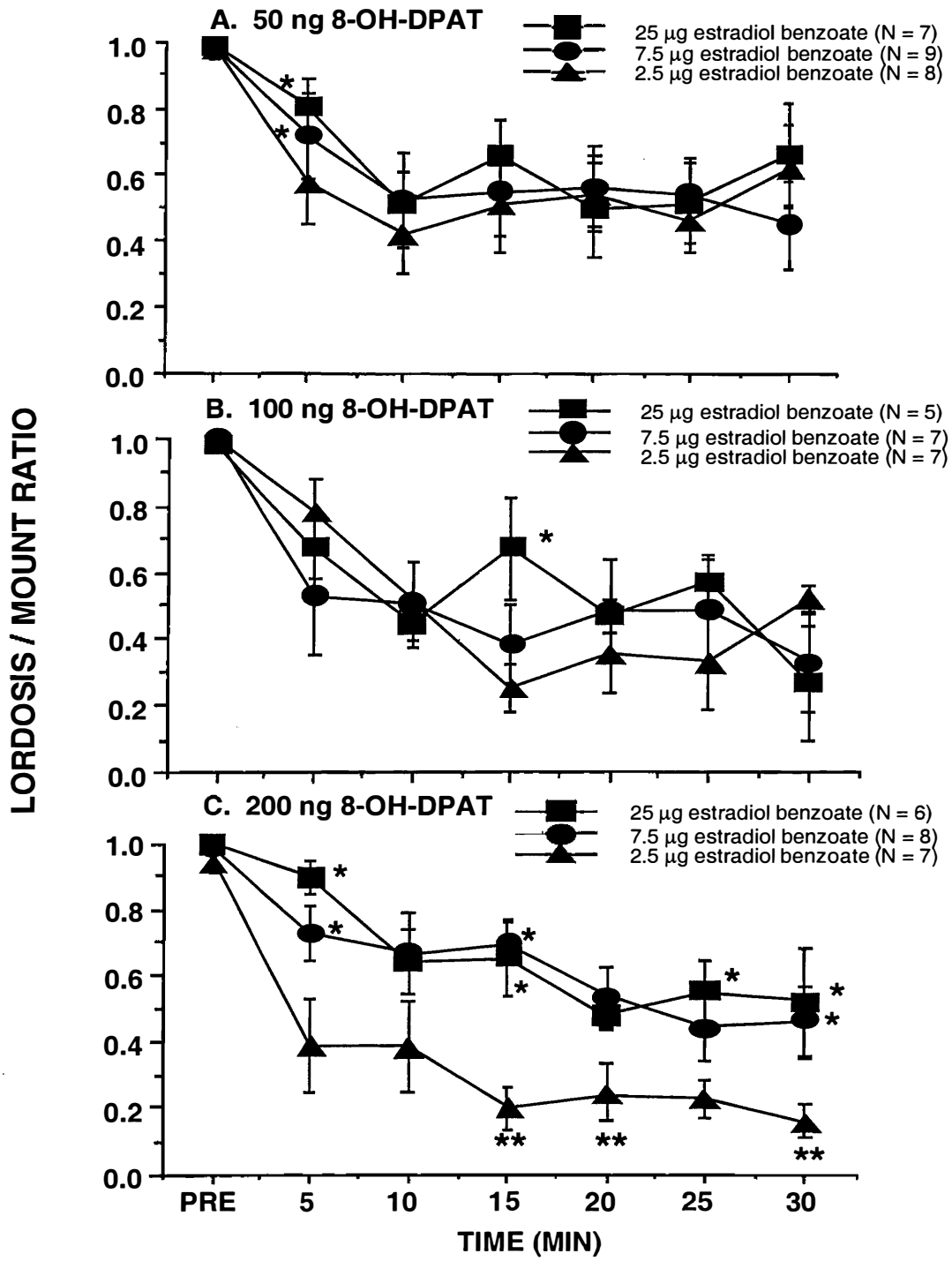
A. 50 ng 8-OH-DPAT
25 µg estradiol benzoate (N = 7)
7.5 µg estradiol benzoate (N = 9)
2.5 µg estradiol benzoate (N = 8)
B. 100 ng 8-OH-DPAT
25 µg estradiol benzoate (N = 5)
7.5 µg estradiol benzoate (N = 7)
2.5 µg estradiol benzoate (N = 7)
C. 200 ng 8-OH-DPAT
25 µg estradiol benzoate (N = 6)
7.5 µg estradiol benzoate (N = 8)
2.5 µg estradiol benzoate (N = 7)
LORDOSIS / MOUNT RATIO
1.0
0.8
0.6
0.4
0.2
0.0
PRE
5
10
15
20
25
30
TIME (MIN)

Figure 5: Dose-dependency of estradiol benzoate and 8-OH-DPAT on lordosis behavior.

Ovariectomized rats, hormone primed with 2.5, 7.5 or 25 μg estradiol benzoate followed 48 hr later with 500 μg progesterone, were infused bilaterally into the VMN with 50 ng (A), 100 ng (B), or 200 ng (C) of 8-OH-DPAT. Data are the mean ± S.E. L/M ratios prior to infusion with 8-OH-DPAT and for 6 consecutive 5-min intervals after infusion. Single asterisks indicate significant differences (within dose of 8-OH-DPAT) from rats hormone-primed with 2.5 μg estradiol benzoate. Double asterisks indicate a significant difference (within priming dose of estradiol benzoate) from rats infused with 50 ng 8-OH-DPAT. N's per treatment condition are indicated in parentheses.

priming conditions. However, the lordosis/mount ratios of rats infused with 200 ng 8-OH-DPAT were significantly lower in rats primed with 2.5 μg than with 25 or 7.5 μg estradiol benzoate, respectively, at 5, 15, 25, and 30 min after infusion and at 5, 15, and 30 min after infusion (Tukey's, $q_{330, 3} \geq 3.31$, $p \leq 0.05$). When priming doses of estradiol benzoate were contrasted, the overall effect of 8-OH-DPAT on the lordosis/mount ratio in rats given 2.5 μg estradiol benzoate was significantly different from that of rats given 7.5 μg or 25 μg of the hormone (F for contrast = 5.53, $p \leq 0.02$). That the drug had a more severe effect in rats primed with 2.5 μg estradiol benzoate was further evidenced by the observation that the lordosis/mount ratio dropped to zero after infusion with 8-OH-DPAT in 11 of the 22 rats treated with the lowest dose of the hormone. In contrast, a zero lordosis/mount ratio was present in only 5 out of 18 and 7 out of 24 of the rats treated with 25 or 7.5 μg estradiol benzoate, respectively.

In agreement with prior studies, there were minimal effects of 8-OH-DPAT on lordosis quality. Because 4 of the rats primed with 2.5 μg estradiol benzoate and infused with 200 ng 8-OH-DPAT had lordosis/mount ratios of zero for a majority of the test session, quality could not be assessed in this group. Consequently, treatment effects on quality were evaluated 1) with every dose of 8-OH-DPAT and the 7.5 and 25 μg doses of estradiol benzoate without the 2.5 μg dose of estradiol benzoate and 2) with every dose of estradiol benzoate and the 50 and 100 ng doses of 8-OH-DPAT. For the first

analysis there was a significant decline in lordosis quality during the 30 min testing period ($F_{6, 186} = 5.03$, $p \leq 0.0001$). There was also a significant interaction between dose of 8-OH-DPAT and time after infusion ($F_{12, 186} = 2.49$, $p \leq 0.005$). When the 200 ng dose of 8-OH-DPAT was excluded from the analysis, there was also a significant decline in lordosis quality after infusion ($F_{6, 174} = 4.62$, $p \leq 0.0002$) and an interaction between time after infusion and dose of 8-OH-DPAT ($F_{6, 174} = 2.05$, $p \leq 0.033$). Data are shown in Figure 6.

FIGURE 6

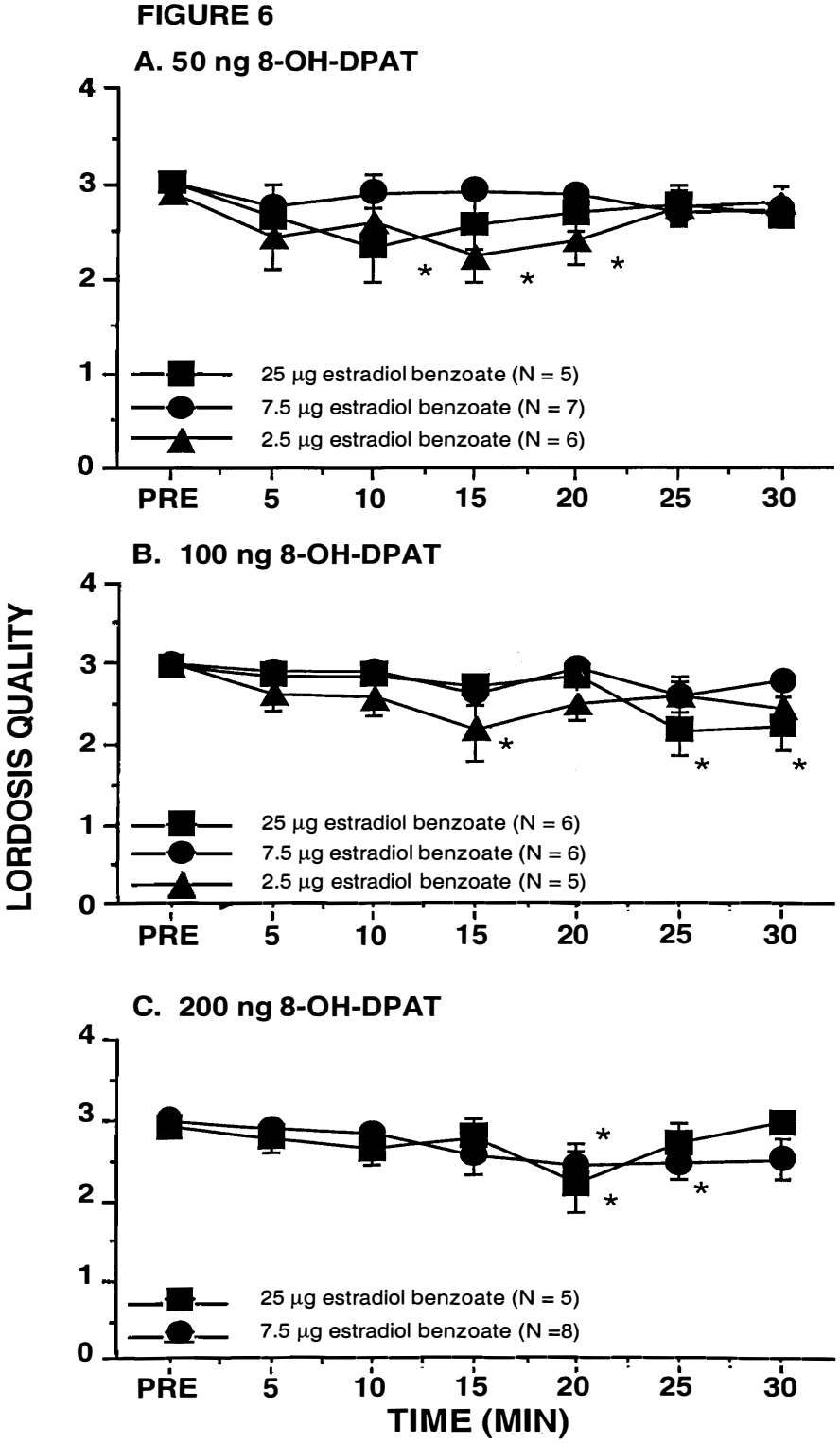
A. 50 ng 8-OH-DPAT
B. 100 ng 8-OH-DPAT
C. 200 ng 8-OH-DPAT
LORDOSIS QUALITY
TIME (MIN)
PRE
5
10
15
20
25
30
0
1
2
3
4
25 µg estradiol benzoate (N = 5)
7.5 µg estradiol benzoate (N = 7)
2.5 µg estradiol benzoate (N = 6)
25 µg estradiol benzoate (N = 6)
7.5 µg estradiol benzoate (N = 6)
2.5 µg estradiol benzoate (N = 5)
25 µg estradiol benzoate (N = 5)
7.5 µg estradiol benzoate (N =8)

Figure 6: Dose-dependency of estradiol benzoate and 8-OH-DPAT on lordosis quality.

Rats were primed with 2.5, 7.5 or 25 μg estradiol benzoate followed 48 hr later with 500 μg progesterone and were infused bilaterally into the VMN with 50 ng (A), 100 ng (B), or 200 ng (C) of 8-OH-DPAT. Shown are the mean ± S.E. lordosis quality scores prior to infusion (PRE) with 8-OH-DPAT and for 6 consecutive 5-min intervals after infusion.

Experiment 4:

Effects of dibutyryl cAMP on the lordosis-inhibiting effects of 8-OH-DPAT.

Because estrogen is known to increase cAMP (Kow et al., 1994) and because the 5-HT_{1A} receptor is negatively coupled to adenylyl cyclase (Zifa and Fillion, 1992), it was hypothesized that an increase in cAMP would resemble prior estrogen treatment and protect against the effects of 8-OH-DPAT.

Rats were pretreated with 25 μg estradiol benzoate (s.c.) and then injected with estradiol benzoate plus progesterone one week later (EEP). Other rats received a single estradiol benzoate plus progesterone hormone priming. Ten of these latter rats were infused with 200 ng 8-OH-DPAT while 9 were coinfused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP. Lordosis/mount ratios are shown in Figure 7A. There was a significant effect of type of treatment ($F_{2, 26} = 26.02$, $p \leq 0.0001$) as well as a significant treatment by time effect on lordosis/mount ratios ($F_{10, 130} = 5.6$, $p \leq 0.0001$). At no time during the 25 min testing period did rats in the EEP treatment group show a significant decline in lordosis/mount ratios (Dunnett's, all $q_{130, 5} \geq 2.44$, $p > 0.05$). Rats that were treated with a single estradiol benzoate injection followed 48 hr later with 500 μg progesterone (EP) showed inhibition of lordosis behavior within 10 min following infusion with 200 ng 8-OH-DPAT and remained inhibited throughout the testing period (Dunnett's, all $q_{130, 5} \geq 2.44$, $p \leq 0.05$). Lordosis/mount ratios for rats treated with estradiol

FIGURE 7

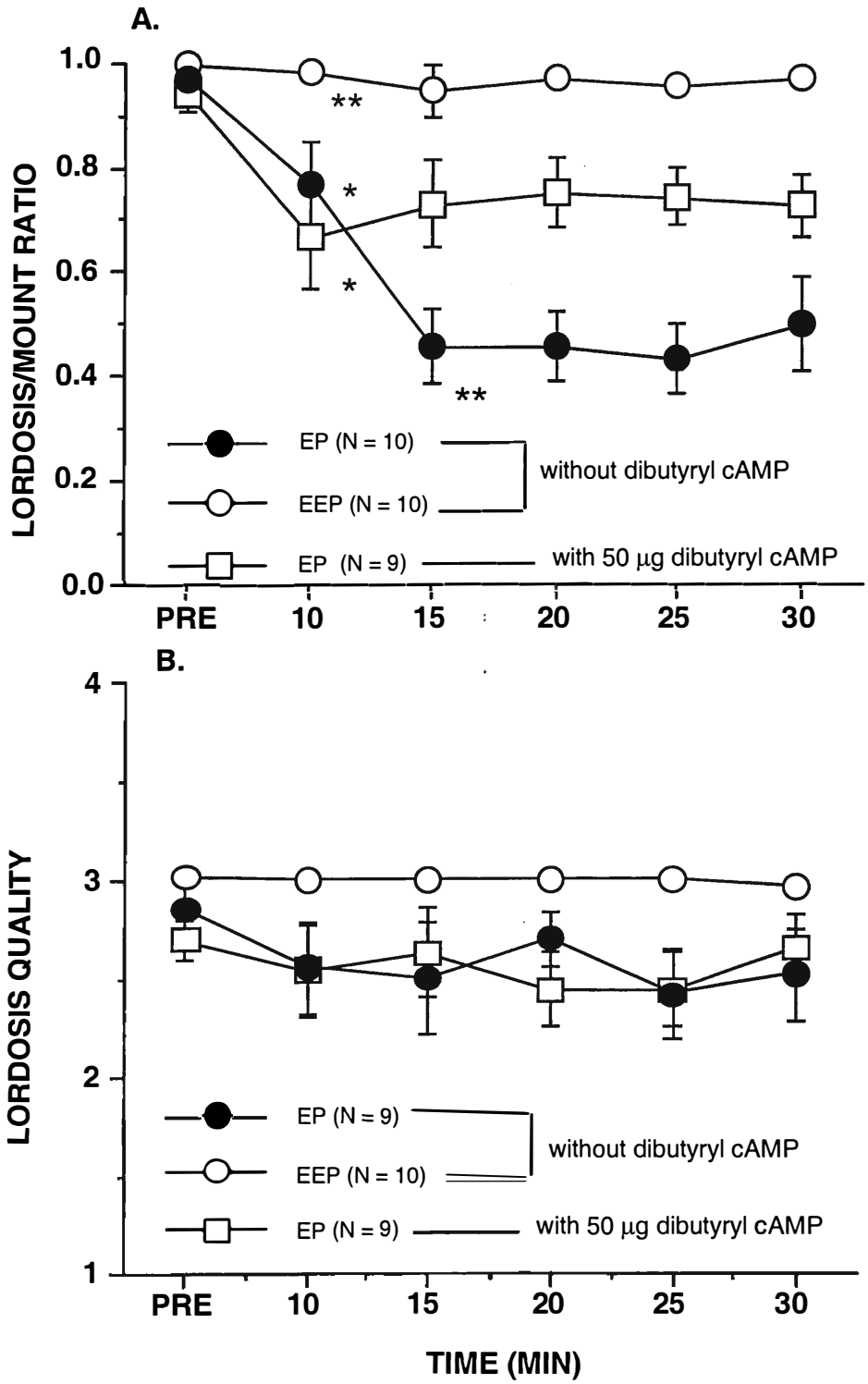
A.
1.0
0.8
0.6
0.4
0.2
0.0
LORDOSIS/MOUNT RATIO
**
*
*
**
EP (N = 10)
EEP (N = 10)
EP (N = 9)
without dibutyryl cAMP
with 50 µg dibutyryl cAMP
PRE
10
15
20
25
30
B.
4
3
2
1
LORDOSIS QUALITY
EP (N = 9)
EEP (N = 10)
EP (N = 9)
without dibutyryl cAMP
with 50 µg dibutyryl cAMP
PRE
10
15
20
25
30
TIME (MIN)

Figure 7: Effects of 8-OH-DPAT plus or minus dibutyryl cAMP into the VMN on lordosis behavior.

Shown in Figure 7A are the mean ± S.E. L/M ratios for rats in three treatment groups. Lordosis quality is shown in Figure 7B. Rats in the EP and EP with dibutyryl cAMP treatment groups received 25 μg estradiol benzoate s.c. followed 48 hr later with 500 μg progesterone. Rats in the EEP treatment group received two estradiol benzoate injections (25 μg s.c.) separated by 7 days. Forty-eight hr after the final estradiol benzoate injection, rats were injected with 500 μg progesterone. All rats were tested for sexual behavior prior to (PRE) and for 25 consecutive min after infusion with the drug(s). Post-infusion testing began 5 min after infusion. Rats in the EP and EEP treatment groups were infused with 200 ng 8-OH-DPAT. N's are 12 and 10, respectively. Rats in the EP with dibutyryl cAMP treatment group were coinfused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP (n = 10). Single asterisks indicate the first 5 min interval during which there was a significant difference from the pretest interval. Double asterisks indicate the first 5 min interval during which there was a significant difference from the EP with dibutyryl cAMP treatment group within the same time interval.

benzoate plus progesterone but infused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP (EP with dibutyryl cAMP) showed a decline in lordosis behavior at 10 min after infusion (Dunnett's, all $q_{130, 5} \geq 2.44$, $p \leq 0.05$). This decline in lordosis behavior lasted throughout the testing period (Dunnett's, all $q_{130, 5} \geq 2.44$, $p \leq 0.05$). Although lordosis/mount ratios of both the EP and EP with dibutyryl cAMP treatment groups declined following 8-OH-DPAT, the decline in the EP with dibutyryl cAMP treatment group was of a lesser magnitude than that seen in the EP treatment group; this difference was present by 15 min and thereafter (Tukey's, $q_{130, 3} \geq 3.31$, $p \leq 0.05$). However, the EEP and EP with dibutyryl cAMP groups were also significantly different from each other by 10 min and thereafter (Tukey's, $q_{130, 3} \geq 3.31$, $p \leq 0.05$). As shown in Figure 7B, there was a minimal effect of 8-OH-DPAT on lordosis quality (all $p > 0.05$).

Mean lordosis/mount ratios and mean lordosis quality scores for rats infused with 50 μg dibutyryl cAMP but without 8-OH-DPAT are shown in Figures 8A and 8B. There was no effect of dibutyryl cAMP on either lordosis/mount ratios or lordosis quality ($p > 0.05$).

FIGURE 8

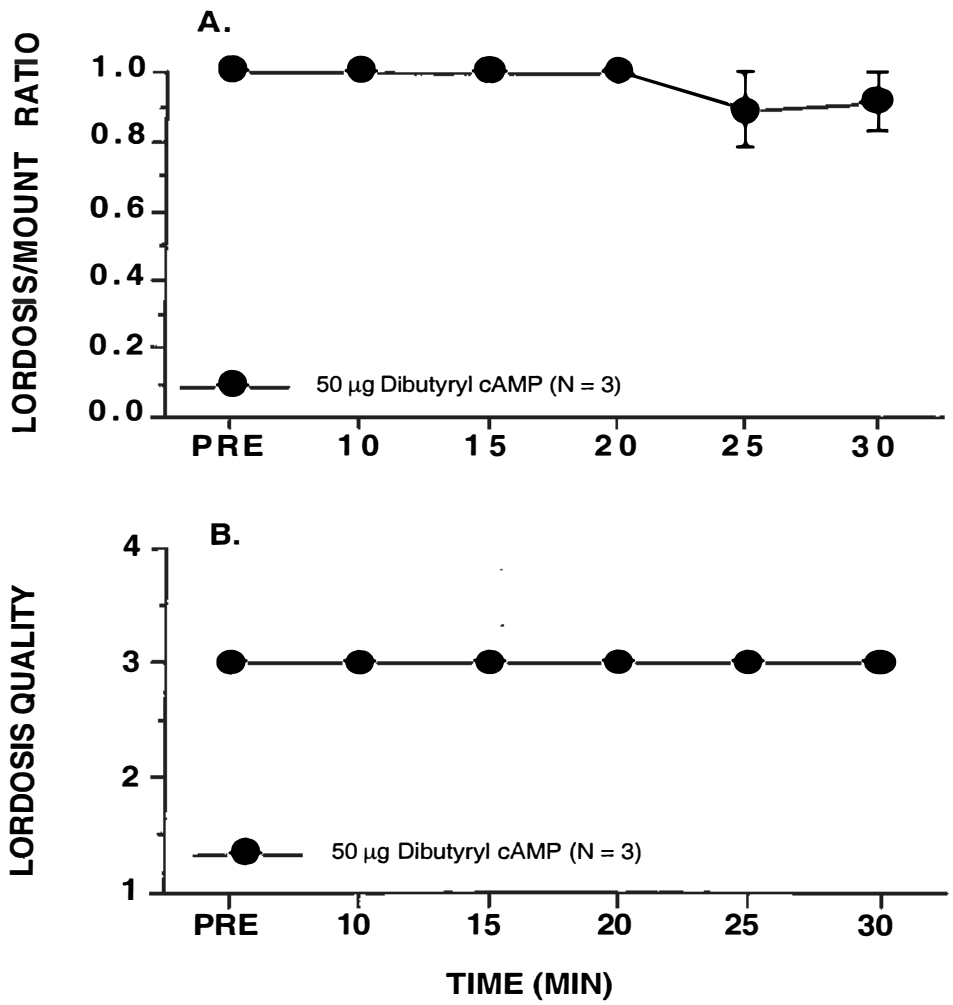

Figure 8: Lack of effect of VMN infusion with dibutyryl cAMP on lordosis behavior.

Three rats were injected with 25 µg estradiol benzoate (s.c.) followed 48 hr later with 500 µg progesterone. Sexual behavior was recorded prior to (PRE) and for 25 consecutive min after infusion with 50 µg dibutyryl cAMP into the VMN. Post-infusion testing began 5 min after infusion. In Figure 8A, the mean ± S.E. L/M ratios are shown. In Figure 8B are shown the mean lordosis quality scores. There was no variation in lordosis quality.

CHAPTER IV

DISCUSSION

The objective of these studies was to evaluate the protective effects of estrogen against the lordosis-inhibiting effects of 8-OH-DPAT. Evidence of modulation of 5-HT_{1A} receptor functioning by estrogen was initially provided by Lakoski (1989). She reported that estrogen reduced the ability of 8-OH-DPAT to reduce firing of dorsal raphe neurons. In our laboratory, we have shown that two estrogen treatments, separated by seven days, reduced the ability of 8-OH-DPAT to inhibit lordosis behavior (Uphouse, et al., 1994). More recently, Jackson and Uphouse (1996) extended our knowledge of 5-HT_{1A} receptor modulation by showing that estrogen treatment reduced the lordosis-inhibiting effects of 8-OH-DPAT within three days of hormone priming. Moreover, a single estrogen treatment reduced the effectiveness of 8-OH-DPAT in inhibiting lordosis behavior when the response to 8-OH-DPAT was evaluated 7 days later (Jackson and Uphouse, 1996).

The present data extend these findings to demonstrate that the protective effects of estrogen persist for at least 10 days after the hormone treatment.

However, after 15 days, the protection from 8-OH-DPAT's effect on lordosis behavior is no longer apparent. Infusion of 17ß-estradiol directly into the VMN followed seven days later by systemic treatment with estradiol benzoate also reduced the lordosis-inhibiting effect of 8-OH-DPAT. Additionally, we report that estrogen can dose-dependently reduce the ability of 8-OH-DPAT to inhibit lordosis behavior on a time scale that is comparable to that required to elicit lordosis behavior. Finally, we report that protection from the lordosis-inhibiting effects of 8-OH-DPAT is also observed when rats are coinfused with 8-OH-DPAT and dibutyryl cAMP. Since activation of VMN 5-HT_{1A} receptors is incompatible with lordosis behavior, these observations are consistent with the suggestion that estrogen's action at 5-HT_{1A} receptors may contribute to the emergence of lordosis behavior. During the time that the behavior is present, the activation of 5-HT_{1A} receptors or their functional impact must be diminished.

It is possible, however, that estrogen has no action at 5-HT_{1A} receptors but that estrogen merely makes the rat more sexually receptive. In view of the fact that hormone priming in ovariectomized rats increases sensitivity to a second hormone priming event (Beach and Orndorff, 1974; Whalen and Nakayama, 1965), the female may be more sexually receptive following the second hormone priming. Increased sensitivity is thought to be due to the changes that occur beyond the time required for facilitation of lordosis behavior. Pfaff and Schwartz-Giblin (1988) postulated that estrogen initiated

a "cascade" of neurochemical events within the VMN and that these hormone-induced changes elicited a readiness for future sexual receptivity that was set in motion by the initial hormonal priming. If so, inhibition of lordosis behavior by 8-OH-DPAT would be less noticeable following the second hormone priming. It is unlikely that increased sensitivity to the second hormone treatment is the mechanism by which estrogen attenuates the inhibitory effect of 8-OH-DPAT on lordosis behavior. Rats given a single estrogen injection followed six days later with progesterone also exhibited a reduced sensitivity to 8-OH-DPAT (Jackson and Uphouse, 1996). As seen in Figure 1, there was no evidence of increased sensitivity to the second hormone treatment 12 days after the initial treatment. When the hormone treatments were separated by 12 days, lordosis behavior was inhibited following injection with 8-OH-DPAT. Additionally, estrogen dose-dependently reduced the ability of 8-OH-DPAT to inhibit lordosis behavior in rats tested for sexual behavior 48 hr after hormone priming (Figure 6).

A second potential explanation for the apparent protection from the lordosis-inhibiting effects of 8-OH-DPAT is an increased sensitivity to progesterone. Increased sensitivity to progesterone is improbable based on experiments in our laboratory during which rats received a single treatment with 25 μg estrogen and 2 mg progesterone. Though the dose of progesterone was increased, estrogen-primed rats still showed a decline in lordosis behavior after infusion of 8-OH-DPAT (Trevino and Uphouse,

unpublished data). Moreover, in rats given two systemic estrogen injections, without progesterone, a reduction in the ability of 8-OH-DPAT to inhibit lordosis behavior was also seen (Truitt and Uphouse, in preparation).

Estrogen's ability to attenuate the effects of 8-OH-DPAT is also unlikely to be due to a decrease in the density of 5-HT_{1A} receptors. Frankfurt et al. (1994) found that after estrogen treatment there was no change in the density of 5-HT_{1A} receptors in the hypothalamus. Similarly, Clarke and Maayani (1990) found no effect of chronic estrogen treatment on the density of 5-HT_{1A} receptors in the hippocampus. Furthermore, higher doses of 8-OH-DPAT have been shown to inhibit lordosis behavior after two hormone treatments (Jackson and Uphouse, 1996). However, we cannot rule out the possibility that a redistribution of 5-HT_{1A} receptors might have occurred as a result of hormone treatment and that the relevant circuitry might have been changed due to an increase/decrease of receptors in some portion of the circuit.

An alternative explanation for the decreased sensitivity to 8-OH-DPAT after estrogen treatment might be a change in receptor coupling. Though the suggestion that estrogen may act to alter synaptic transmission of G-protein coupled receptors throughout the brain has been made (LaGrange, Ronnekeiv and Kelly, 1997), to date there is no evidence of estrogen acting at G-proteins. However, it has been shown that pretreatment of cultured striatal neurons with 17ß-estradiol enhanced the pertussis toxin-catalyzed ADP-ribosylation of Gαo,i proteins (Maus, Homburger, Bockert, Glowinski, and Premont, 1990).

It is possible that estrogen treatment leads to a decrease in 5-HT release. In a study using cycling female rats, Maswood et al. (1998) showed that microdialysate concentrations of 5-HT declined in proestrous rats. In the estrogen-treated rat, progesterone appears to be required for the decline in 5-HT (Farmer et al., 1996).

A final consideration is that estrogen may act to accentuate mechanisms that are facilitatory to lordosis behavior. Agonists at 5-$HT_{2A/2C}$ receptors facilitate lordosis behavior (Mendelson and Gorzalka, 1986) and 5-HT_{2C} receptors, in particular, may be responsible for the facilitation (Wolf, et al., 1998). Both 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors may be located on the same neurons and interaction between the receptors has been suggested (Zifa and Fillion, 1992). Following estrogen treatment, an increase in 5-HT_2 receptor density has been shown (Beigon et al., 1983). It has also been shown that estradiol stimulated an increase in the amount of 5-HT_{2A} receptor mRNA (Sumner and Fink, 1995).

Estrogen treatment has also been shown to increase cAMP (Aronica, Kraus and Katzenellenbogen, 1994) and events that increase cAMP increase lordosis behavior (Kow et al., 1994). Beyer, et al. (1981) demonstrated that dibutyryl cAMP could facilitate lordosis behavior in ovariectomized estrogen-primed rats. The current data would be consistent with this possibility. Thus, modulation by estrogen of other events that attenuate the effects of activation of 5-HT_{1A} receptors may account for the reduced sensitivity to the 5-HT_{1A} agonist.

In summary, the present findings demonstrate that the protection from the lordosis-inhibiting effects of 8-OH-DPAT occurs for approximately 10 days after 25 μg estradiol benzoate. At least one site of estrogen action is the VMN because 17ß-estradiol directly into the VMN protected against 8-OH-DPAT's inhibition of lordosis behavior. Estrogen's mechanism has not yet been delineated but probably involves changes in overall consequences to 8-OH-DPAT action at VMN neurons. The protective action of dibutyryl cAMP against the effects of 8-OH-DPAT is consistent with this view. These results are consistent with the hypothesis that activation of 5-HT_{1A} receptors or their functional impact are diminished as the female moves from the sexually non-receptive state to the sexually receptive state. Future studies will be required to elucidate the precise mechanisms involved.

REFERENCES

Ahlenius, S.N., Fernandez-Gausti, A., Hijoth, S. and Larsson, K. (1986). Suppression of lordosis behavior by the putative 5-HT receptor agonist 8-OH-DPAT in the rat. *Eur. J. Pharm.* **124:** 361-363.

Aiello-Zaldivar, M., Luine, V. and Frankfurt, M. (1992). 5,7-DHT facilitated lordosis: effects of 5-HT agonists. *Neuroreport* **3:** 542-544.

Aronica, S.M., Kraus, W.L. and Katzenellenbogen, B.S. (1994). Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc. Natl. Acad. Sci.* **91:** 8517-8521.

Barfield, R.J., and Chen, J.J. (1977). Activation of estrous behavior in ovariectomized rats by intracerebral implants of estradiol benzoate. *Endocrinology* **101:** 1716-1725.

Beach, F.A. (1976). Sexual attractivity, proceptivity and receptivity in female mammals. *Horm. Behav.* **7:** 105-138.

Beach, F.A., and Orndoff, R.K. (1974). Variations in the responsiveness of female rats to ovarian hormones as a function of preceding hormonal deprivation. *Horm. Behav.* **5:** 201-205.

Beyer, C., Canchola, E. and Larsson, K. (1981). Facilitation of lordosis behavior in the ovariectomized estrogen primed rat by dibutyryl cAMP. *Physiol. Behav.* **26:** 249-251.

Biegon, A., Fischette, T., Rainbow, T.C. and McEwen, B.S. (1982). Serotonin receptor modulation by estrogen in discrete brain nuclei. *Neuroendocrinology* **35:** 287-291.

Biegon, A. and McEwen, B.S. (1982). Modulation by estradiol of serotonin1 receptors in brain. *J. Neurosci.* **2:** 199-205.

Biegon, A., Reches, A., Snyder, L. and McEwen, B.S. (1983). Serotonergic and noradrenergic receptor modulation by chronic exposure to ovarian hormones. *Life Sci.* **32:** 2015-2021.

Bockaert, J., Dumuis, A., Bouhelal, R., Sebben, M. and Cory, R.N. (1987). Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists of 5-HT_{1A} receptors negatively coupled with adenylate cyclase in hippocampal neurons. *Naunyn Schmiedebergs Arch Pharmacol.* **335:** 588-592.

Clarke, W.P. and Maayani, S. (1990). Estrogen effects on 5-HT_{1A} receptors in hippocampal membranes from ovariectomized rats: functional and binding studies. *Brain Res.* **518:** 287-291.

Cohen, R.S. and Pfaff, D.W. (1981). Ultrastructure of neurons in the ventromedial nucleus of the hypothalamus in ovariectomized rats with or without estrogen treatment. *Cell Tiss. Res.* **217:** 451-470.

DeCamilli, P., Macconi, D. and Spada, A. (1979). Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas. *Nautre* **278:** 252-254.

DeVivo, M. and Maayani, S. (1985). Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists. *Eur. J. Pharmacol.* **119:** 231-234.

Endersby, C.A. and Wilson, C.A. (1974). The effects of ovarian steroids on the accumulation of ^{3}H-labelled monoamines by hypothalamic tissue in vitro. *Brain Res.* **73:** 321-331.

Fadem, B.H., Barfield, R.J. and Whalen, R.E. (1979). Dose-response and time-response relationships between progesterone and the display of patterns of receptive and proceptive behavior in the female rat. *Horm. Behav.* **13:** 40-48.

Farmer, C.J., Isakson, T.R., Coy, D.J. and Renner, K.J. (1996). In vivo evidence for progesterone dependent decreases in serotonin release in the hypothalamus and midbrain central grey: relation in the induction of lordosis. *Brain Res.* **711:** 84-92.

Feder, H.H. (1984). Hormones and sexual behavior. *Annu. Rev. Psychol.* **35:** 165-200.

Frankfurt, M., McKittrick, C.R., Mendelson, S.D. and McEwen, B.S. (1994). Effects of 5,7-dihydroxytryptamine, sex and gonadal steroids on serotonin receptor binding in rat brain. *Neuroendocrinology* **59:** 245-250.

Grierson, J.P., James, M.D., Pearson, J.R. and Wilson, C.A. (1988). The effects of selective D1 and D2 dopaminergic agents on sexual receptivity in the female rat. *Neuropharm.* **27:** 181-189.

Hoyer, D. and Schoeffter, P. (1991). 5-HT receptors: Subtypes and second messengers. *J. Recept. Res.* **11:** 197-214.

Jackson, A. and Uphouse, L. (1996). Prior treatment with estrogen attenuates the effects of the 5-HT_{1A} agonist, 8-OH-DPAT, on lordosis behavior. *Horm. Behav.* **30:** 145-152.

Kebabian, J.W., Petzold, G.L. and Greengard, P. (1972). Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and it's similarity to the "dopamine receptor." *Proc. Natl. Acad. Sci. USA* **69:** 2145-2149.

Konig, J. and Klippel. R. (1963). The Rat Brain. A Sterotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Williams and Wilkins, Baltimore.

Kow, L-M., Mobbs, C.V. and Pfaff, D.W. (1994). Roles of second-messenger systems and neuronal activity in the regulation of lordosis by neurotransmitters, neuropeptides and estrogen: A review. *Neurosci. Biobehav. Rev.* **18:** 251-268.

Lagrange, A.H., Ronnekleiv, O.K., Kelly, M.J. (1997). Modulation of G protein-coupled receptors by an estrogen receptor that activates protein kinase A. *Mol. Pharmacol.* **51:** 605-612.

Lakoski, J.M. (1989). Cellular electrophysiological approached to the central regulation of female reproductive aging. *In* J . M. Lakoski, J. R. Perez-Polo, and D. K. Rassin, (Eds.)*, Neurology and Neurobiology. Neural Control of Reproductive Function,* Vol. 50, pp. 209-220. Alan R. Liss, Inc., New York.

Maswood, S., Hotema, M., Caldarola-Pastuszka, M. and Uphouse, L. (1998) Estrous cycle modulation of extracellular serotonin in the mediobasal hypothalamus: role of serotonin transporter and terminal autoreceptors. In preparation.

Matthews, D. and Edwards, D.A., (1977). Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. *Physiol. Behav.* **19:** 319-326.

Maus, M., Homburger, V., Bockaert, J., Glowinski, J. and Premont, J. (1990). Pretreatment of mouse striatal neurons in primary culture with 17 beta-estradiol enhances the pertussis toxin-catalyzed ADP-ribosylation of G alpha o,i protein subunits. *J. Neurochem.* **55:** 1244-1251.

McCarthy M.M., Kaufman L.C., Brooks, P.J., Pfaff, D.W. and Schwartz-Giblin, S. (1995). Estrogen modulation of mRNA levels for the two forms of glutamic acid decarboxylase (GAD) in female rat brain. *J Comp. Neurol.* **360:** 685-697.

McQueen, J.K., Wilson, H. and Fink, G. (1997). Estradiol-17 beta increases serotonin transporter (SERT) mRNA levels and the density of SERT-binding sites in female rat brain. *Mol. Brain Res.* **45:** 13-23.

Meisel, R.L. and Pfaff, D.W. (1985). Specificity and neural sites of action of anisomycin in the reduction or facilitation of female sexual behavior in rats. *Horm. Behav.* **19:** 237-251.

Menard, C.S., Herbert, T.J., Ross, S.M. and Dohanich, G.P. (1992). The effect of estrogen treatment on scopolamine inhibition of lordosis. *Horm. Behav.* **26:** 364-374.

Mendelson, S.D. and Gorzalka, B.B. (1986). Serotonin type 2 antagonists inhibit lordosis behavior in the female rat: reversal with quipazine. *Life Sci.* **38:** 33-39.

Pfaff, D.W., Lewis, C., Diakow, C. and Keiner, M. (1972). Neurophysiological analysis of mating behavior responses as hormone-sensitive reflexes. In *Progress in Physiological Psychology*, eds. E. Stellar and J.M. Sprague, Vol. 5. pp. 253-297. Academic Press, New York.

Pfaff, D.W. and Modianos, D. (1985). Neural mechanisms of female behavior. In *Handbook of Behavioral Neurobiology,* eds. N. Adler, D. W. Pfaff, and R. W. Goy, pp. 423-493. Plenum Press, New York.

Pfaff, D.W. and Sakuma,Y. (1979). Facilitation of the lordosis reflex in female rats from the ventral medial nucleus of the hypothalamus. *J. Physiol.* **288:** 189-202.

Pfaff, D.W., and Schwartz-Giblin, S. (1988). Cellular mechanisms of female reproductive behavior. *In* E. Knobil, and J. Neill, (Eds.), *The Physiology of Reproduction,* pp. 1487-1568. Raven Press, New York.

Pleim, E.T. and Barfield, R.J. (1988). Progesterone versus estrogen facilitation of female sexual behavior by intracranial administration to female rats. *Horm. Behav.* **22:** 150-159.

Powers, J.B. (1970). Hormonal control of sexual receptivity during the estrous cycle of the rat. *Physiol. Behav.* **5:** 8310835.

Rubin, B.S. and Barfield, R.J. (1980). Priming of estrous responsiveness by implants of 17 beta-estradiol in the ventromedial hypothalamic nucleus of female rats. *Endocrinology* **106:** 504-509.

Sumner, B.E.H., Rosie, R. and Fink, G. (1992). Relative density of 5-hydroxytryptamine receptor subtype mRNAs in female rat neuroendocrine brain determined by *in situ* hybridization histochemistry. *Mol. Cell. Neurosci.* **3:** 215-223.

Sumner, B.E.H. and Fink, G. (1993). Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. *Mol. Cell. Neurosci.* **4:** 83-92.

Sumner, B.E.H. and Fink, G. (1995). Estrogen increases the density of 5-hydroxytraptamine (2A) receptors in cerebral cortex and nucleus accumbens in the female rat. *J Steroid Biochem. Mol. Biol.* **54:** 15-20.

Sumner, B.E.H. and Fink, G. (1997). The density of 5-hydroxytryptamine 2A receptors in forebrain is increased at pro-oestrus in intact female rats. *Neurosci. Lett.* **234:** 7-10.

Trevino, A., Wolf, A., Jackson, A., Price, T. and Uphouse, L. (1998). Reduced efficacy of 8-OH-DPAT's inhibition of lordosis behavior by prior estrogen treatment. Submitted.

Truitt, W. and Uphouse, L. (1998). Progesterone does not alter the effects of 8-OH-DPAT on female rat lordosis behavior. In preparation.

Uphouse, L., Salamanca, S. and Caldarola-Pastuszka, M. (1991a). Gender and estrous cycle differences in the response to the 5-HT_{1A} agonist, 8-OH-DPAT. *Pharmacol. Biochem. Behav.* **40:** 901-906.

Uphouse, L., Montanez, S., Richards-Hill, R., Caldarola-Pastuszka, M. and Droge, M. (1991b). Effects of 8-OH-DPAT on sexual behavior of the proestrous rat. *Phamacol. Biochem. Behav.* **39:** 635-640.

Uphouse, L., Caldarola-Pastuszka, M. and Montanez, S. (1992). Intracerebral action of the 5-HT_{1A} agonists, 8-OH-DPAT and buspirone, and of the 5-HT_{1A} partial agonist/antagonist, NAN-190, on female sexual behavior. *Neuropharmacology* **31:** 969-981.

Uphouse, L., Caldarola-Pastuszka, M., Maswood, S., Andrade, M. and Moore, N. (1994). Estrogen-progesterone and 8-OH-DPAT attenuate the lordosis-inhibiting effects of the 5-HT_{1A} agonist in the VMN. *Brain Res.* **637:** 173-180.

Uphouse, L., Maswood, S., Jackson, A., Sinclair-Worley, L. and Caldarola-Pastuszka, M. (1997). Intracerebral infusion with forskolin protects against the effects of 8-OH-DPAT on lordosis behavior. Soc. Neurosci. Abst. **23:** 519.

Vogel, S.A., Janowsky, D.S. and Davis, J.M. (1970). Effects of estradiol on stimulus-induced release of ^{3}H-noreprinephrine and ^{3}H serotonin from rat brain slices. *Res. Commun. Chem. Path. Pharm.* **1:** 451-459.

Whalen, R.E. (1974). Estrogen-progesterone induction of mating in female rats. *Horm. Behav.* **5:** 157-162.

Wolf, A., Caldarola-Pastuszka, M. and Uphouse, L. (1998). Facilitation of female rat lordosis behavior by hypothalamic infusion of 5-HT2A/2C receptor agonists. *Brain Res.* **779:** 84-95.

Yocca, F.D. and Maayani, S.D. (1990). 5-HT receptors linked to adenylyl cyclase activity in mammalian brain. *Ann. N.Y. Acad. Sci.* **600:** 213-223.

Zar, J.H. (1996). Biostatistical Analysis, third edition, Prentice Hall, New Jersey.

Zifa, E. and Fillion, G. (1992). 5-Hydroxytryptamine Receptors. *Pharm. Reviews* **44:** 3, 401-458.