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Estrogen modulates 5-HT_{1A} agonist inhibition of lordosis behavior but not binding of [^{3}H]-8-OH-DPAT

Astra Jackson, Anne M. Etgen*

Department of Neuroscience, F113, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

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Abstract

Previous studies showed that repeated estrogen treatment reduces the ability of the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-*n*-propylamino) tetralin (8-OH-DPAT), to inhibit lordosis behavior of female rats. The present study evaluated the effects of repeated estrogen treatment on lordosis behavior and 5-HT_{1A} receptor binding and coupling to G protein in the hypothalamus-preoptic area using the agonist ligand [^{3}H]-8-OH-DPAT, which binds selectively to G-protein-coupled 5-HT_{1A} receptors. Rats were injected twice with 25 or 50 μg of estradiol benzoate (EB) 7 days apart followed by 500 μg of progesterone (P) 48 h after the second EB injection. Controls received a single injection of 25 or 50 μg EB followed 48 h later by 500 μg of P. Four hours after P, 0.15 mg/kg 8-OH-DPAT was injected, and lordosis behavior examined for 30 min. Rats treated twice with EB showed significantly less 8-OH-DPAT inhibition of lordosis behavior than rats receiving a single EB injection. For receptor binding, rats received EB without P treatment. None of the estrogen treatments reduced [^{3}H]-8-OH-DPAT binding density or affinity in the hypothalamus-preoptic area or hippocampus. These studies suggest that estrogen modulates 5-HT_{1A} agonist potency without a measurable change in 5-HT_{1A} receptor density or coupling to G protein. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: 5-HT_{1A} receptor; G protein coupling; Lordosis behavior; Hippocampus; Hypothalamus; Estrogen

1. Introduction

The estrogen-dependent lordosis posture is a supraspinal reflex exhibited by female rodents in response to the male's mounting behavior (Pfaff and Modianos, 1985). Estrogen is essential for the elicitation of the reflex (Clemens and Weaver, 1985). Removal of estrogen by ovariectomy causes a dramatic reduction in the display of the copulatory posture, which can be restored by estrogen replacement (Powers, 1970). The behavior is further enhanced by administration of progesterone to estrogen-primed females (Fadem et al., 1979; Whalen, 1974).

Serotonin (5-HT) receptors also play a role in lordosis behavior (Uphouse, 2000). Depending upon receptor subtype, activation of 5-HT receptors may facilitate or inhibit lordosis behavior (Ahlenius et al., 1986; Uphouse et al., 1992; Wolf et al., 1998). Activation of the 5-HT_{1A} receptor subtype by systemic administration of 5-HT_{1A} agonists inhibits lordosis behavior (Ahlenius et al., 1986; Mendelson and Gorzalka, 1986). Infusion of 5-HT_{1A} agonists into one of the areas thought to be essential for estrogen facilitation of reproductive behavior, the ventromedial nucleus of the hypothalamus (VMN), also inhibits lordosis behavior in proestrous and in ovariectomized, hormone-primed female rats (Uphouse et al., 1991).

Initial evidence suggesting that estrogen modulates 5-HT_{1A} function was provided by Lakoski (1989), who reported that estrogen reduced the ability of the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-*n*-propylamino) tetralin (8-OH-DPAT), to reduce firing of dorsal raphe neurons. Consistent with the view that estrogen reduces 5-HT_{1A} function are studies that examined inhibition of lordosis behavior after estrogen priming (Jackson and Uphouse, 1996, 1998; Trevino et al., 1999; Uphouse et al., 1994a). Following a single treatment with 25 μg of estradiol benzoate (EB) and 500 μg of progesterone (P), inhibition of lordosis behavior was seen after VMN infusion of 8-OH-DPAT (Jackson and Uphouse, 1996; Trevino et al., 1999). In contrast, when rats were given two estrogen treatments

* Corresponding author. Tel.: +1-718-430-3662; fax: +1-718-430-8654.

E-mail address: etgen@aecom.yu.edu (A.M. Etgen).

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separated by 7 days, then followed by P, the same dose of 8-OH-DPAT was significantly less effective at inhibiting lordosis behavior.

Although early experiments utilizing ligands that do not discriminate among 5-HT_1 receptor subtypes suggested that estrogen decreased the density of 5-HT_1 binding sites in the hypothalamus (Biegon et al., 1982), recent reports have offered conflicting data on estrogen modulation of 5-HT_{1A} receptor binding. In ovariectomized rats primed with EB plus P to induce receptive behavior (lordosis quotient 80–100%), neither the density nor binding affinity of 5-HT_{1A} receptors in micropunches of the VMN, preoptic area, anterior hypothalamus, median eminence and arcuate nucleus differed from control rats when measured by [^{3}H]-8-OH-DPAT binding (Gonzalez et al., 1997). Flugge et al. (1999), using quantitative in vitro receptor autoradiography with [^{3}H]-8-OH-DPAT, reported an increase in VMN binding sites in proestrous and estrous rats compared to diestrous rats. Additionally, [^{3}H]-8-OH-DPAT binding decreased after ovariectomy and was restored to levels equivalent to estrous females 3 days after implantation of Silastic capsules containing estradiol. These authors reported no change in receptor affinity for [^{3}H]-8-OH-DPAT across the estrous cycle (Flugge et al., 1999). Most authors have focused on a hormone-priming regime that is consistent with the timescale for lordosis induction. The present study analyzed effects of repeated estrogen treatments that demonstrably alter sensitivity to 5-HT_{1A} receptor agonists. Thus, in this report, we examined whether prior exposure to EB could alter 5-HT_{1A} receptor binding in the female rat hypothalamus and preoptic area using [^{3}H]-8-OH-DPAT. This ligand has been reported to bind selectively to the agonist high-affinity state, which is believed to represent G-protein-coupled 5-HT_{1A} receptors (Assie et al., 1999). Therefore, use of this ligand should provide information about receptor G protein coupling.

2. Methods

2.1. Animals

Sprague–Dawley female rats purchased from Taconic Farms (Germantown, NY) were housed two or three per cage on a 14–10 h reversed light–dark cycle with ad lib access to food and water. Rats were anesthetized with halothane (Halocarbon Labs, River Edge, NJ) and bilaterally ovariectomized. At the conclusion of the experiment, ovariectomy was confirmed by postmortem examination.

2.2. Sexual behavior

Steroid hormones EB and P were purchased from Steraloids (Wilton, NH) and dissolved in peanut oil. Eighteen rats received one of the following hormone treatments: one injection with either 25 or 50 μg EB followed 48 h later by 500 μg of P (EP-25 or EP-50, $n=4$ per group), or two injections, separated by 7 days with either 25 or 50 μg EB followed 48 h later by 500 μg of P (EEP-25 or EEP-50, $n=5$ per group). In both behavioral and binding experiments, EB and P were administered subcutaneously in a volume of 0.1 ml of peanut oil.

Behavioral testing with 8-OH-DPAT (Research Biochemicals, Natick, MA) began 4 h after P. Prior to injection with 8-OH-DPAT, females were placed in a 15-gal observation chamber with a sexually experienced male, and sexual behavior was recorded for a minimum of 10 mounts. The female was then removed, injected intraperitoneally with 0.15 mg/kg 8-OH-DPAT, and returned to the observation chamber. The drug dose was chosen on the basis of extensive prior work by the Uphouse laboratory as one that reliably and reversibly inhibits lordosis without causing the flattened body posture often associated with 5-HT agonists (Jackson and Uphouse, 1996, 1998; Trevino et al., 1999; Uphouse et al., 1994a). Lordosis behavior was recorded for the next 30 min. Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (i.e., number of lordosis responses by the female divided by the number of mounts by the male). L/M ratios were computed for the pretest and for each of six 5-min intervals after injection as described by Uphouse et al. (1991). Upon termination of lordosis testing, rats were placed in an open field and tested for locomotion (quantified as line crossing) and rearing behavior for 5 min to verify that 8-OH-DPAT did not impair motor function.

2.3. Tissue preparation

For binding assays rats were treated with a standard estrogen-priming dose, 2 μg of EB given 24 and 48 h before sacrifice. In our laboratory, more than 90% of ovariectomized female rats demonstrate L/M ratios between 0.8 and 1.0 when this estradiol treatment regimen is followed by 200–500 μg of P. Other rats were given 25 or 50 μg of EB 48 h before sacrifice. The final groups were given two injections, separated by 7 days, of either 25 or 50 μg of EB with the final injection given 48 h before sacrifice. The latter conditions were chosen to mimic the hormone priming conditions in which rats show reduced sensitivity to 8-OH-DPAT during lordosis behavior testing (Fig. 1). Ovariectomized control rats were injected with oil vehicle. All animals were sacrificed by rapid decapitation. The brain was removed and the hypothalamus-preoptic area and hippocampus were dissected and placed separately in 2 ml of ice-cold buffer containing 50 mM Tris and 10 mM $MgCl_2$ (pH 7.4). Hypothalamus-preoptic area tissue from two rats given the same hormone treatment was pooled to provide sufficient material for saturation analysis. The homogenates were centrifuged at $40{,}000 \times g$ for 20 min at 4°C. The supernatant was removed and the pellet resuspended in 2 ml Tris buffer. This was repeated four times. The pellet was then suspended in 2 ml Tris buffer and incubated at 37°C for 20 min in a shaking water bath to remove endogenous 5-HT

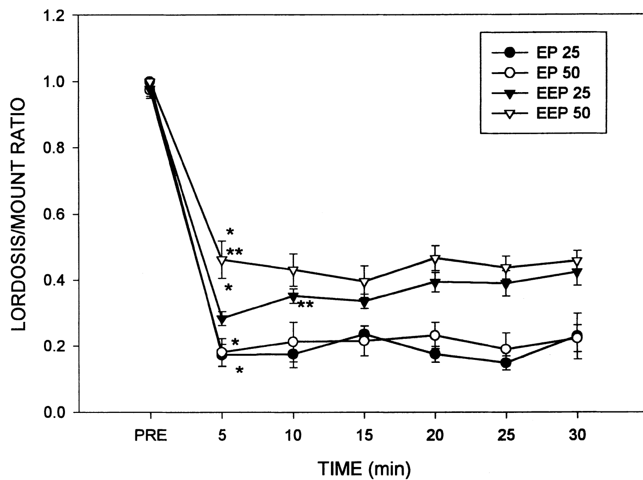

Fig. 1. Effects of 0.15 mg/kg 8-OH-DPAT (ip) on lordosis behavior. Rats were given one injection with either 25 or 50 μg EB followed 48 h later by 500 μg of P (EP-25 or EP-50), or two injections, separated by 7 days with either 25 or 50 μg EB followed 48 h later by 500 μg of P (EEP-25 or EEP-50). Data are the mean ± S.E.M. lordosis/mount (L/M) ratios before injection (PRE) and for each 5-min interval after injection with 0.15 mg/kg 8-OH-DPAT. *N*'s respectively were 4, 4, 5 and 5. * $P<.05$ vs. the pretest. ** $P<.05$ (EP-25 or EP-50) vs. (EEP-25 or EEP-50). Single and double asterisks indicate first interval of significant difference. Groups remained different throughout the 30-min test period.

(Nelson et al., 1978). Following a final centrifugation (40,000 × *g*; 20 min; 4°C), pellets were stored at − 70°C until assayed.

2.4. Binding assay

The agonist ligand 8-OH-DPAT is known to bind only to the agonist high-affinity state of the 5-HT_{1A} receptor, which represents the G-protein-coupled state of the receptor. This property of 8-OH-DPAT is demonstrated by the ability of nonhydrolyzable guanine nucleotide analogs to displace 8-OH-DPAT binding to 5-HT_{1A} receptors (Assie et al., 1999; Clarke and Maayani, 1990; Khawaja et al., 1995, 1997). In agreement with published reports, we found that GppNHp displaced [^{3}H]-8-OH-DPAT binding in membrane homogenates from hypothalamus-preoptic area and hippocampus (data not shown). Therefore, [^{3}H]-8-OH-DPAT binding provides a measure of receptor–G protein coupling. The affinity (K_d) and total density (B_{max}) of 8-OH-DPAT binding were measured by saturation binding methods followed by Scatchard analysis. The ligand [^{3}H]-8-OH-DPAT, ranging in concentration from 0.05–5 nM (124.9 Ci/mmol, New England Nuclear, Boston, MA), was incubated with membrane aliquots, in triplicate, for 1 h at 25°C. Nonspecific binding was determined by the addition of 5 μM 5-HT (Research Biochemicals). Binding was performed in buffer containing 50 mM Tris, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$ (pH 7.4) (Khawaja et al., 1995, 1997). Bound radioligand was separated from free by rapid vacuum filtration over Whatman GF/B filters using a Brandel cell harvester. Filters were presoaked for 1 h in ice-cold Tris buffer. Filters were washed three times with ice-cold Tris buffer and placed in scintillation vials containing 4 ml of scintillation fluid and counted in a Beckman LS3801 counter. Protein content was determined by the Lowry method (Lowry et al., 1951).

2.5. Data analysis

Behavioral data were evaluated by two-way ANOVA with hormone treatment as the independent factor and time after 8-OH-DPAT as the repeated factor. Tukey's test was used to determine differences between hormone treatment groups. Dunnett's test was used to compare pretest behavior to behavior after 8-OH-DPAT injection within treatment groups. Binding data were analyzed using one-way ANOVA. An alpha level of .05 was required for rejection

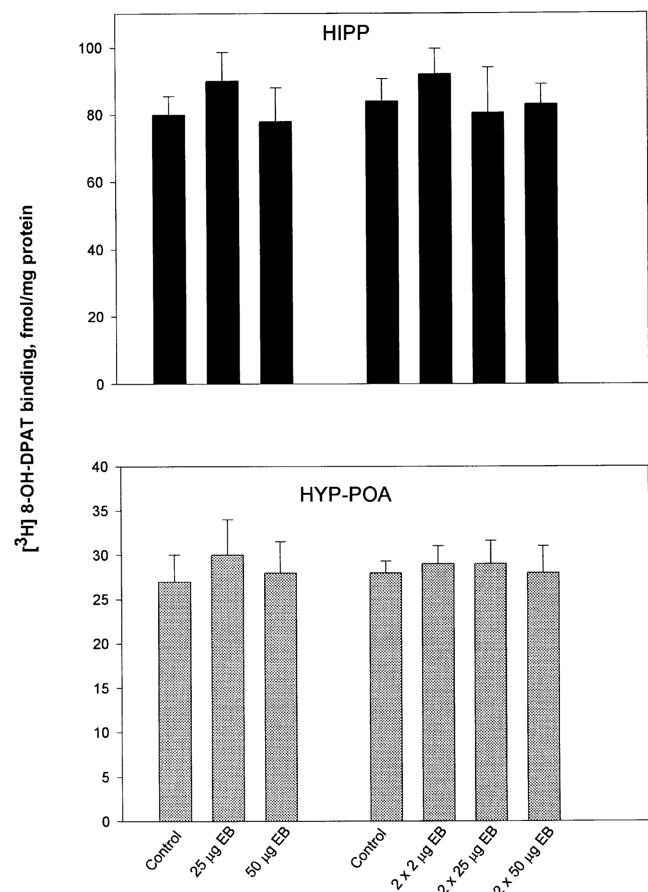

Fig. 2. Effect of hormone treatment on [^{3}H]-8-OH-DPAT binding density in the hypothalamus-preoptic area (HYP-POA) (lower panel) and hippocampus (HIPP) (upper panel). Rats in the left three bars were treated with 25 or 50 μg EB 48 h before sacrifice (25 or 50 μg EB). Repeated estrogen treatments (shown on the right) were 2 μg of EB given 24 and 48 h before sacrifice (2 × 2 μg EB), or two injections separated by 7 days, with 25 or 50 μg EB (2 × 25 or 2 × 50 μg EB) with a final injection given 48 h before sacrifice. Data are the results of saturation binding studies followed by Scatchard analysis. Values represent the mean ± S.E.M. of three to four independent replications.

Table 1
The effects of estrogen treatment on [^{3}H]-8-OH-DPAT affinity for 5-HT$_{1A}$ receptors

	Receptor affinity (K_d, nM)						
	Control	25 μg EB	50 μg EB	Control	2 × 2 μg EB	2 × 25 μg EB	2 × 50 μg EB
HYP-POA	1.5 ± 0.4	1.5 ± 0.2	1.8 ± 0.1	1.4 ± 0.1	1.4 ± 0.06	1.6 ± 0.3	1.5 ± 0.4
HIPP	0.66 ± 0.03	0.68 ± 0.07	0.63 ± 0.04	0.62 ± 0.03	0.68 ± 0.01	0.6 ± 0.02	0.65 ± 0.05

Each value represents the mean ± S.E.M. of three to four independent replications. Data were analyzed by one-way ANOVA. There was no difference between control and estrogen treatment groups.

of the null hypothesis. These experiments were conducted in accordance with the NIH *Guide for Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee.

3. Results

3.1. Lordosis behavior

Previous studies using Fisher (CDF) rats have shown that the inhibitory effects of 8-OH-DPAT on lordosis behavior are significantly reduced in rats primed with two injections of 25 μg of EB separated by 7 days. However, when rats are given a single 25 μg injection of EB, robust inhibition of lordosis behavior is observed within 5 min of 8-OH-DPAT administration (Trevino et al., 1999; Uphouse et al., 1994b). Current data in Sprague–Dawley rats are consistent with these findings. ANOVA showed a significant effect of hormone treatment, time after injection with 8-OH-DPAT and Time × Hormone treatment interaction (all $P < .05$). As seen in Fig. 1, within 5 min of 8-OH-DPAT administration, rats that received a single EB injection (EP 25 or EP 50) showed a decrease in lordosis behavior relative to their pretest (Dunnett's, $P < .05$). The reduction in lordosis behavior persisted throughout the entire 30-min testing period (Dunnett's, all $P < .05$). The 5-HT$_{1A}$ agonist also significantly reduced lordosis behavior in rats treated with two EB injections followed by P (EEP 25 or EEP 50; Dunnett's, $P < .05$ vs. pretest). When L/M ratios of the EP treatment groups were compared to EEP treatment groups, lordosis behavior in the EEP treatment groups was significantly higher than in the EP groups (Tukey's, $P < .05$). The EP 25 treatment group was significantly different from the EEP 25 treatment group beginning 10 min after injection with 8-OH-DPAT and lasting for the remainder of the 30 min test period (Tukey's, $P < .05$). Within 5 min of 8-OH-DPAT injection, the EP 50 treatment group was significantly different from the EEP 50 treatment and remained so for the entire 30 min test period (Tukey's, $P < .05$). Females in all groups received between 10 and 12 mounts per 5-min test interval, and there was no evidence that 8-OH-DPAT impaired motor function. No animal demonstrated flattened body posture at any time after drug administration. In a subset of animals ($n = 2$ per treatment group) in which open field activity was scored, locomotion scores ranged from 59 to 72 lines crossed and 27 to 36 rearing events per 5 min.

3.2. [^{3}H]-8-OH-DPAT binding

To assess the effect of low-dose estrogen treatment on [^{3}H]-8-OH-DPAT binding, we treated rats with 2 μg of EB 24 and 48 h before sacrifice. This priming regime modulates the density and G protein coupling of several noradrenergic receptors in the hypothalamus (Karkanias and Etgen, 1994; Petitti et al., 1992; Ungar et al., 1993). We also investigated the higher, single and multiple doses of EB utilized in this and previous lordosis behavior studies in which reduced behavioral sensitivity to the 5-HT$_{1A}$ agonist, 8-OH-DPAT, are observed (Jackson and Uphouse, 1996; Trevino et al., 1999; Uphouse et al., 1994b). Treatment with 2 μg of EB 24 and 48 h before sacrifice had no effect on [^{3}H]-8-OH-DPAT binding density in the hypothalamus-preoptic area or hippocampus as compared to oil controls (Fig. 2). There was also no effect on the K_d (receptor affinity) of [^{3}H]-8-OH-DPAT binding following this treatment in either brain region examined (Table 1). Similarly, neither treatment for 48 h with 25 or 50 μg EB nor repeated treatment with 25 or 50 μg EB altered receptor density (Fig. 2) or receptor affinity (Table 1) in either of the brain regions examined.

4. Discussion

In agreement with previous studies in Fischer rats, Fig. 1 shows that 8-OH-DPAT inhibits lordosis behavior in Sprague–Dawley rats primed with a single injection of EB, followed 48 h later by 500 μg of P. Moreover, repeated estrogen priming reduces the ability of 8-OH-DPAT to inhibit lordosis behavior in this rat strain. We hypothesized that this change in behavioral sensitivity to 8-OH-DPAT is accompanied by a change in 5-HT$_{1A}$ receptor density, G protein coupling, or affinity in the hypothalamus-preoptic area. Although behavioral response to 8-OH-DPAT is affected by repeated priming with high doses of estradiol, we find no evidence of changes in receptor density or affinity in the hypothalamic-preoptic area. Thus, changes in 5-HT$_{1A}$ receptor density or affinity are not obligatory for the reduced ability of 5-HT$_{1A}$ agonists to inhibit lordosis behavior. Although we cannot entirely rule out the possibility that changes in 5-HT$_{1A}$ receptor binding might have been detected if the hypothalamus and preoptic area had been analyzed separately, we believe this is unlikely. Previous studies that used receptor autoradiography (e.g., Gonzalez et al., 1997) did not report estrogen-induced

changes in [^{3}H]-8-OH-DPAT binding in either the hypothalamus or preoptic area. Indeed, in agreement with our work, most studies report no change in either receptor affinity or density, despite variation in the doses and duration of estrogen treatment (e.g., Frankfurt et al., 1994; Gonzalez et al., 1997). The only exception is a recent study (Flugge et al., 1999) in which estradiol given by Silastic capsule for 3 days moderately increased rather than decreased 8-OH-DPAT binding in the VMN without any change in the preoptic area.

We also found that none of the estrogen treatments modifies 5-HT$_{1A}$ receptor density or affinity in the hippocampus. Therefore, our results are consistent with several previous studies in which estrogen treatments did not modify 5-HT$_{1A}$ binding. For example, Clarke and Maayani (1990) reported that 4 days of estrogen treatment via Silastic capsule did not alter [^{3}H]-8-OH-DPAT binding in the hippocampus, even though 5-HT$_{1A}$ agonist inhibition of adenylyl cyclase was enhanced. Similarly, Frankfurt et al. (1994) found no change in binding of [^{3}H]-8-OH-DPAT to 5-HT$_{1A}$ receptors in the VMN following administration of two daily injections of 10 μg of EB with or without 500 μg of P. Lower doses of EB (2 μg given 48 h before assay) also fail to alter 8-OH-DPAT binding in the hypothalamus or preoptic area (Gonzalez et al., 1997). However, our data differ from those of Osterlund et al. (2000), who report a decrease in binding of the 5-HT$_{1A}$ receptor antagonist, [^{3}H] WAY 100635, in the hippocampus after 2 week treatment with estradiol. These authors found no changes in 5-HT$_{1A}$ receptor mRNA expression after estradiol treatment. This group used Silastic implants to maintain elevated estradiol levels throughout the 2-week treatment and utilized receptor autoradiography to assess [^{3}H] WAY 100635 binding. Thus, methodological differences could account for our divergent results in hippocampus. Unfortunately, the hypothalamus was not examined in the Osterlund study.

A reduction in 5-HT$_{1A}$ receptor coupling to G proteins could reduce the ability of 8-OH-DPAT to inhibit lordosis behavior. A recent report (Mize and Alper, 2000) suggests such a possibility. The investigators examined 5-HT$_{1A}$ receptor coupling to G proteins using agonist stimulation of [^{35}S]-GTPλS binding and report that estrogen treatment reduced coupling in the cortex, hippocampus and amygdala of female rats measured 2 h after estradiol treatment. However, this reduction was not evident following estradiol administration for 14 days via Silastic capsules. Another recent study reported that 10 μg of EB reduced the concentration of Gz, Gi$_1$, and Gi$_3$ proteins in the hypothalamus (Raap et al., 2000). As noted previously, [^{3}H]-8-OH-DPAT has been shown to preferentially recognize the agonist high affinity binding state of 5-HT$_{1A}$ receptors in hippocampal membrane preparations (Clarke and Maayani, 1990; Khawaja et al., 1995) and of recombinant human 5-HT$_{1A}$ receptors stably expressed in Chinese hamster ovary cells (Khawaja et al., 1997). In agreement with these reports, we find that the nonhydrolyzable guanine nucleotide analog GppNHp displaces most [^{3}H]-8-OH-DPAT binding in female rat brain membranes (data not shown). Hence, the failure of repeated estrogen treatment to reduce [^{3}H]-8-OH-DPAT binding in the hypothalamus-preoptic area suggests that estrogen does not modify behavioral sensitivity to 8-OH-DPAT by uncoupling 5-HT$_{1A}$ receptors from G protein.

Other neurotransmitter receptors known to facilitate or inhibit lordosis behavior can be modulated by estrogen (Hebert et al., 1994; Menard et al., 1992; Priest et al., 1995). There is considerable evidence that estrogen regulates β- and α_2-noradrenergic receptors by uncoupling them from G proteins (Karkanias and Etgen, 1994; Ungar et al., 1993). Functionally, estrogen also attenuates presynaptic α_2-adrenoceptor inhibition of norepinephrine release (Karkanias and Etgen, 1993) and β-adrenoceptor activation of adenylyl cyclase (Ungar et al., 1993) in the hypothalamus. Because β-adrenoceptor activation can inhibit lordosis and α_2-adrenoceptor activation attenuates norepinephrine release, functional uncoupling of these receptors by estradiol is thought to contribute to hormonal facilitation of lordosis behavior in female rats. The present results suggest that estrogen attenuates 5-HT$_{1A}$ inhibition of lordosis by mechanism(s) different from its actions on adrenergic receptors in the hypothalamus and preoptic area.

Although we find no evidence that 5-HT$_{1A}$ receptors are reduced or uncoupled by estrogen treatment, the density of 5-HT$_{2A/2C}$ receptors, which facilitate lordosis behavior (Mendelson and Gorzalka, 1985), increases in response to estrogen (Sumner and Fink, 1995). Estrogen treatment also increases 5-HT$_{2A}$ receptor density and receptor mRNA in cerebral cortex and nucleus accumbens (Sumner and Fink, 1995). Moreover, the density of 5-HT$_{2A/2C}$ binding sites is higher on the afternoon of proestrous than on the morning of diestrus in frontal and cingulate cortex and in nucleus accumbens (Sumner and Fink, 1997).

Estrogen may also modulate G-protein-coupled receptor signaling by regulating the enzymes producing or metabolizing second messengers involved in lordosis behavior. Estrogen treatment increases cAMP levels in uterus of Sprague–Dawley rats and in MCF-7 human breast cancer cells (Aronica et al., 1994), and elevation of hypothalamic cAMP levels can increase lordosis behavior (Kow et al., 1994). Infusion of dibutyryl-cAMP or 8-bromo-cAMP into the VMN of proestrous rats also protects against the lordosis-inhibiting effects of 8-OH-DPAT (Uphouse, 2000). These data suggest that repeated estrogen administration could reduce behavioral sensitivity to 8-OH-DPAT by modulating the levels of second messengers that facilitate lordosis behavior.

In summary, we have demonstrated that repeated estrogen treatment attenuates the effects of the 5-HT$_{1A}$ agonist, 8-OH-DPAT, on lordosis behavior. However, there was no evidence of a reduction in 5-HT$_{1A}$ receptor binding affinity, 5-HT$_{1A}$ receptor number or G protein coupling as measured by [^{3}H]-8-OH-DPAT in membrane homogenates of the hypothalamus-preoptic area. These

studies suggest that estrogen modulates 5-HT_{1A} agonist potency without a measurable change in 5-HT_{1A} receptor coupling to G protein.

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