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Prevention of Progesterone-Induced Lordosis Behavior by Alpha or Beta Adrenergic Antagonists in Ovariectomized Estrogen-Primed Rats

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FERNÁNDEZ-GUASTI, A., K. LARSSON AND C. BEYER. *Prevention of progesterone-induced lordosis behavior by alpha or beta adrenergic antagonists in ovariectomized estrogen-primed rats.* PHARMACOL BIOCHEM BEHAV 22(2) 279–282, 1985.—The effect of systemic administration of adrenergic alpha (phenoxybenzamine and prazosin) and beta (propranolol) antagonists on the lordosis behavior induced by progesterone (2.0 mg/rat) was studied in ovariectomized estradiol benzoate (4.0 μg/rat) primed rats. The effect of these antagonists was also tested on the lordosis behavior induced in ovariectomized rats by estradiol benzoate alone (1.25 μg/rat each two days). Phenoxybenzamine (0.8, 4.0 and 20.0 mg/kg), propranolol (0.8, 4.0 and 20.0 mg/kg) and prazosin (0.2 and 1.0 mg/kg) caused a dose-dependent reduction of progesterone induced lordosis. By contrast, phenoxybenzamine (4.0 mg/kg), propranolol (4.0 mg/kg) or prazosin (1.0 mg/kg) did not affect estrogen induced lordosis behavior. Results suggest the following conclusions: (1) blockage of either alpha or beta adrenoreceptors prevents progesterone induced lordosis behavior and (2) the adrenergic neuron involved in progesterone facilitation of lordosis behavior is not a part of the reflex arc for lordosis but probably modulates the activity of this system.

Phenoxybenzamine Propranolol Prazosin Progesterone-induced lordosis

LORDOSIS behavior is dependent on the sequential action of estrogen and progesterone (P) in the brain [16]. Our understanding of the mechanism by which these steroids interact to stimulate lordosis is limited. Indirect evidence suggests that estrogen acts by stimulating protein synthesis [1] but the mechanism of P action is uncertain. We have recently proposed [1] that one of the key molecular events induced by P to stimulate lordosis is a rise in cAMP levels leading to the phosphorylation and activation of proteins previously induced by estrogen. This model of P action is supported by the following experimental data: (1) P increases cAMP levels in the hypothalamus of ovariectomized estrogen-primed rats (Collado and Beyer, unpublished data), (2) administration of cAMP analogues [2] and adenylate cyclase activators [4,10] induce lordosis and, (3) treatment with phosphodiesterase inhibitors potentiate the effect of P on lordosis in ovariectomized estrogen-primed rats [3].

There is no evidence that P acts directly on membrane receptors linked to an adenylate cyclase-cAMP system. On the contrary, recent data show that adrenergic beta blockers interfere with the P effect on hypothalamic cAMP levels (Collado and Beyer, unpublished data). Therefore, it appears likely that P facilitates lordosis by favouring the release or the action of norepinephrine (NE). This idea is supported by the following observations: (1) P induces NE release both *in vivo* [17] and *in vitro* [13], (2) NE and epinephrine (E) induce lordosis when infused into the ventromedial hypothalamic (VMH) nucleus of estrogen primed rats [11] and, (3) NE and E act on the hypothalamus by raising cAMP levels [20].

Therefore, in the present study we decided to test the effect of various adrenergic blockers, both alpha (phenoxybenzamine or prazosin) and beta (propranolol), on (1) the facilitatory effect on lordosis behavior exerted by P in estrogen-primed rats and (2) on the lordosis behavior induced by repeated injections of estradiol benzoate to ovariectomized rats. This last experiment was made in order to determine whether these antagonists act by interfering with the adrenergic transmission in the reflex arc for lordosis.

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METHOD

GENERAL METHODS

Animals

Female Wistar rats (250–300 g body wt) bred and maintained in our laboratory were used in this study. The rats housed in a temperature controlled (24°C) room under inverted light-dark cycle (14 hr light-10 hr dark) were fed with Purina rat chow and water available ad lib. The rats were ovariectomized using ether anesthesia three weeks before drug treatment.

Drugs

Estradiol benzoate and progesterone (purchased from Sigma Chemical Co.) were dissolved in oil and SC injected. Phenoxybenzamine (a gift of Smith, Kline and French) was dissolved in dichloromethane and thereafter in oil and injected SC. Propranolol (purchased from Sigma Chemical Co.) was dissolved in saline and injected IP. Prazosin (a gift of Pfizer) was dissolved in acetic acid 100% and 5% glucose-saline, buffered to a pH of 7.4 with a solution of NaOH 2 M and injected IP.

Behavioral Tests

Tests were conducted in a circular Plexiglas arena, 50 cm diameter. Sires were vigorous experienced males. During the test, each female received a total of 10 mounts with pelvic thrusts. Receptivity was quantified by a lordosis quotient (LQ=number of lordosis/10 mounts × 100). Data were statistically compared using the Kruskal-Wallis analysis of variance followed by the Mann Whitney U test or by the Wilcoxon T test [22].

EXPERIMENT 1. EFFECT OF ADRENERGIC ANTAGONISTS ON ESTROGEN-PROGESTERONE INDUCED LORDOSIS BEHAVIOR

Rats received initially a subcutaneous injection of 4.0 μg/rat of estradiol benzoate (EB) dissolved in 0.2 ml of sesame oil, and were injected 44 hr later with 2.0 mg/rat P dissolved in 0.2 ml of sesame oil. Two hours after P treatment, the animals were injected with either phenoxybenzamine or propranolol. Additional groups were treated with prazosin, administered 1 hour after P. Controls were injected with vehicle 2 hours after P treatment. Tests for lordosis behavior were conducted at 4 and 8 hr after P injection. The following groups and treatments were used in this experiment:

Group 1. 2.0 ml/kg vehicle, 7 rats; Group 2. 0.16 mg/kg phenoxybenzamine, 7 rats; Group 3. 0.8 mg/kg phenoxybenzamine, 6 rats; Group 4. 4.0 mg/kg phenoxybenzamine, 7 rats; Group 5. 20.0 mg/kg phenoxybenzamine, 7 rats; Group 6. 0.04 mg/kg prazosin, 7 rats; Group 7. 0.2 mg/kg prazosin, 6 rats; Group 8. 1.0 mg/kg prazosin, 6 rats; Group 9. 0.16 mg/kg propranolol, 7 rats; Group 10. 0.8 mg/kg propranolol, 7 rats; Group 11. 4.0 mg/kg propranolol, 8 rats; Group 12. 20.0 mg/kg propranolol, 7 rats.

EXPERIMENT 2. EFFECT OF ADRENERGIC ANTAGONISTS ON ESTROGEN INDUCED LORDOSIS BEHAVIOR

Animals in this experiment were injected every other day with 1.25 μg/rat EB. In order to establish baseline levels for lordosis behavior, the rats were tested daily for lordosis and animals showing a LQ of 70 or higher were selected for experimental treatment. The LQs established in the pretest were considered as controls for each experimental treatment. Following the pretest, animals were injected with either phenoxybenzamine (4.0 mg/kg), propranolol (4.0 mg/kg) or prazosin (1.0 mg/kg) in dosages found to be effective in preventing the effect of P in Experiment 1. Rats were tested for lordosis behavior 2 and 6 hours (phenoxybenzamine and propranolol) or 3 and 7 hours (prazosin) after drug administration. Hours of observation in this experiment correspond to hours of observation after drug treatment in Experiment 1.

RESULTS

EXPERIMENT 1. EFFECT OF ADRENERGIC ANTAGONISTS ON ESTROGEN-PROGESTERONE INDUCED LORDOSIS BEHAVIOR

Control rats show a LQ of 71 and 80 at 4 and 8 hours respectively after P administration. Treatment with phenoxybenzamine (Fig. 1) or propranolol (Fig. 2) in dosages larger than 0.8 mg/kg, or prazosin (Fig. 3) in dosages larger than 0.2 mg/kg caused a dose-dependent reduction of lordosis behavior. Administration of 0.16 mg/kg of either phenoxybenzamine or propranolol (Figs. 1 and 2, respectively) or of 0.04 mg/kg prazosin (Fig. 3) was followed by a non-signficiant reduction of the LQ. These results show that treatment with either alpha or beta adrenergic blockers prevents P induced lordosis behavior. None of the dosages of the drugs used in this experiment caused other behavioral alterations.

EXPERIMENT 2. EFFECT OF ADRENERGIC ANTAGONISTS ON ESTROGEN INDUCED LORDOSIS BEHAVIOR

Table 1 shows the results of this experiment. Phenoxybenzamine, propranolol or prazosin at dosages that effectively prevent P-induced lordosis behavior, (Experiment 1) did not affect lordosis induced by repeated injections of EB.

DISCUSSION

Present results demonstrate that treatment with either alpha or beta adrenergic antagonists prevents, in a dose dependent manner, the display of lordosis induced by P in estrogen primed rats (Experiment 1). By contrast, these adrenergic antagonists did not affect the ongoing lordosis behavior induced by repeated injections of estradiol benzoate (Experiment 2). The fact that adrenergic antagonists interfere with estrogen-progesterone-, but not with ongoing estrogen-, induced lordosis behavior, indicates that these agents do not act at synapses of the reflex arc for lordosis, but rather interfere with the facilitatory activity of a system influenced by P. The possibility that both alpha and beta adrenergic blockers act as antiprogestins, e.g., interfering with the binding of P to its receptors, appears unlikely. A more plausible explanation is that these agents act on a noradrenergic system activated or modulated by P administration.

Surprisingly, few studies have tried to prevent the action of P on lordosis by administering adrenergic antagonists. Recently, Collado, Rodríguez-Manzo and Beyer (unpublished data) in our laboratory found that propranolol (a beta antagonist) could depress P induced lordosis in rats, a finding in agreement with the present study. Davis and Kohl [9] reported that phenoxybenzamine, an alpha blocker, given 4 hours after P, failed to block the ongoing lordosis behavior in the rat. This last result contrasts with our observation showing that phenoxybenzamine prevents the display of lordosis

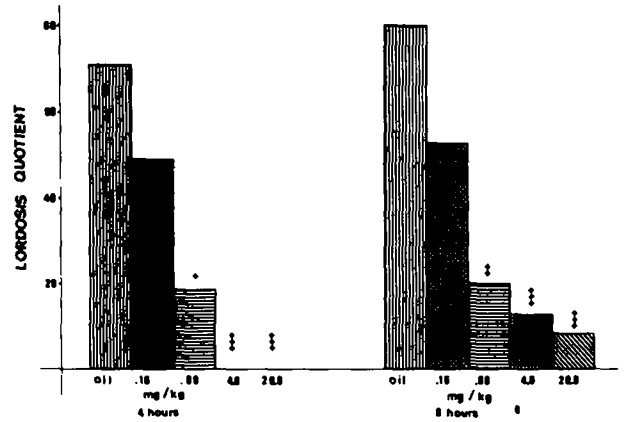

FIG. 1. Effect of phenoxybenzamine, administered 2 hr after progesterone, on the lordosis behavior induced by estradiol benzoate (4.0 μg/rat) followed 44 hr later by progesterone (2.0 mg/rat) in ovariectomized rats. Bars represent mean lordosis quotient after oil or different dosages of phenoxybenzamine. Hours indicate time of observation after progesterone administration. Mann Whitney U test †$p<0.05$, ††$p<0.01$, †††$p<0.001$. Kruskal Wallis analysis of variance $p<0.001$.

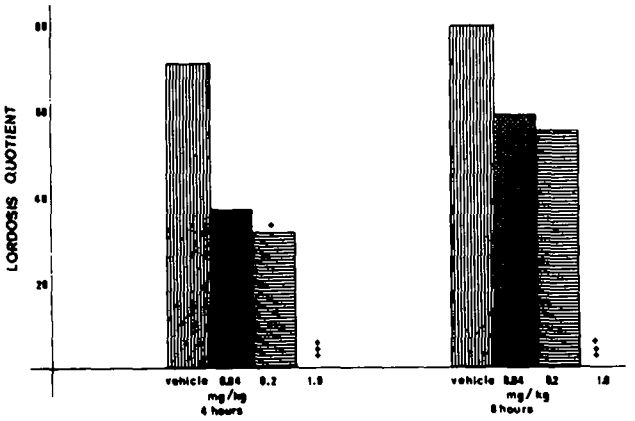

FIG. 3. Effect of prazosin, administered 1 hr after progesterone, on the lordosis behavior induced by estradiol benzoate (4.0 μg/rat) followed 44 hr later by progesterone (2.0 mg/rat) in ovariectomized rats. Bars represent mean lordosis quotient after vehicle or different dosages of prazosin. Hours indicate time of observation after progesterone administration. Mann Whitney U test †$p<0.05$, †††$p<0.001$. Kruskal Wallis analysis of variance $p<0.01$.

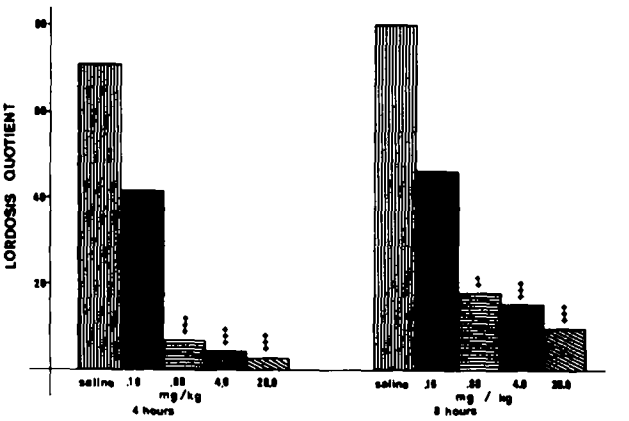

FIG. 2. Effect of propranolol, administered 2 hr after progesterone, on the lordosis behavior induced by estradiol benzoate (4.0 μg/rat) followed 44 hr later by progesterone (2.0 mg/rat) in ovariectomized rats. Bars represent mean lordosis quotient after saline or different dosages of propranolol. Hours indicate time of observation after progesterone administration. Mann Whitney U test ††$p<0.01$, †††$p<0.001$. Kruskal Wallis analysis of variance $p<0.01$.

TABLE 1

EFFECT OF ALPHA (PHENOXYBENZAMINE AND PRAZOSIN) OR BETA (PROPRANOLOL) ADRENERGIC BLOCKING AGENTS ON THE LORDOSIS BEHAVIOR INDUCED BY ESTRADIOL BENZOATE (1.25 μg/RAT EACH TWO DAYS) IN OVARIECTOMIZED RATS

Treatment	n	Lordosis Quotient mean ± S.D.
A. Phenoxybenzamine (4.0 mg/kg)	6	
Pretest		83 ± 12
2 hr after drug		82 ± 13
6 hr after drug		78 ± 20
B. Prazosin (1.0 mg/kg)	6	
Pretest		86 ± 15
3 hr after drug		86 ± 10
7 hr after drug		80 ± 19
C. Propranolol (4.0 mg/kg)	6	
Pretest		81 ± 12
2 hr after drug		88 ± 12
6 hr after drug		85 ± 13

No statistical differences were found using Wilcoxon T test.

when given 2 hours after P. This discrepancy may be due to the different time schedules used to interfere with the effect of P, since at 4 hours lordosis is already expressed. These data suggest that the noradrenergic neurons do not participate directly in the expression of lordosis but, induce changes in the excitability of the reflex arc for lordosis behavior occurring before lordosis is overtly displayed.

The finding that both alpha and beta adrenergic blockers interfere with the stimulatory effect of P on lordosis appears somewhat puzzling. Yet, these results agree well with several biochemical and pharmacological studies on the mechanism of action of NE in the hypothalamus. Both alpha and beta agonists can increase cAMP levels in the rat hypothalamus [6,23]. Moreover, according to Daly *et al.* [6] who studied the effect of various adrenergic agonists and antagonists on brain cAMP levels, "the hypothalamus is unique in being the only brain region in which potentiative interactions between alpha and beta adrenergic mechanisms were found to be significant." This peculiar condition of the hypothalamus can explain why either alpha or beta antagonists can block adrenergic responses in the hypothalamus [20], and strongly suggest that functions mediated by E or NE in this brain region should be depressed by either alpha or beta antagonists. Indeed, phenoxybenzamine and propranolol can inhibit intraspecies aggressive behavior in isolated mice [7,25] and, as shown in the present study, can prevent estrogen-P induced lordosis behavior.

Implantation and uptake studies [12,21] indicate that P acts on VMH neurons to stimulate lordosis. This area appears to be the site of interaction of P and NE, since adrenergic fibers arising in the lateral tegmentum establish synaptic contacts with dendrites of the VMH neurons [5,14] and E or NE infusion into this nucleus stimulates lordosis in estrogen primed rats [11]. Although the above studies point to the VMH as the site for steroid catecholamine interaction, the precise cellular mechanisms involved in the process are uncertain. Nock and Feder [18,19] have proposed a model for the interaction between steroids and NE to induce lordosis behavior in the guinea pig. This model suggests that NE activity increases the number of interacellular P receptors, a condition favouring the display of lordosis in estrogen primed females [15]. Although this mechanism may also operate in the rat, an alternative model in which P acts at a membrane level modulating noradrenergic transmission can also be proposed from the following data: (1) P facilitates adrenergic and noradrenergic transmission in the peripheral nervous system [8], (2) P receptors have been recently described in synaptosomes of rat brain [24], (3) P induces hypothalamic NE release [17] and (4) P increases NE passive efflux and inhibits NE reuptake in rat brain synaptosomes [13]. Further studies, however, are necessary to establish the precise mechanism through which sex steroids and catecholamines interact to facilitate lordosis behavior.

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