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Blockade of LHRH-Induced Lordosis by α- and β-Adrenergic Antagonists in Ovariectomized, Estrogen Primed Rats

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GONZALEZ-MARISCAL, G. AND C. BEYER. *Blockade of LHRH-induced lordosis by α- and β-adrenergic antagonists in ovariectomized, estrogen primed rats.* PHARMACOL BIOCHEM BEHAV 31(3) 573–577, 1988.—The participation of a noradrenergic mechanism in the facilitation of lordosis by luteinizing hormone-releasing hormone (LHRH) was studied in two groups of ovariectomized estrogen primed rats, with or without sexual experience. The administration of 5 μg estradiol benzoate (EB) alone to sexually inexperienced subjects (Ss) induced weak lordosis behavior in some of them (mean lordosis quotient, LQ=12±19). The SC injection of 5 μg LHRH significantly increased this response four hours later (LQ=38±41), though great variability was observed (59% of Ss showing LQs below 30). The systemic administration of either prazosin, an α-adrenergic antagonist (0.2 or 1 mg/kg), or propranolol, a β-adrenergic antagonist (20 mg/kg), totally suppressed LHRH-induced lordosis in sexually inexperienced Ss (mean LQs=8±11; 5±10; 18±31, respectively). In sexually experienced Ss (tested on two previous occassions with EB and LHRH) the administration of EB alone on a third test induced significant levels of lordosis (mean LQ=51±41). The administration of 5 μg LHRH to sexually experienced, estrogen primed Ss induced near maximal levels of lordosis (LQ=94±18). In these Ss, prazosin (0.2 and 1 mg/kg) and, to a lesser extent, propranolol (20 mg/kg) significantly depressed lordosis to values that were not significantly different from those obtained after EB alone (mean LQs=59±38; 63±20; 74±32, respectively). These results indicate that blockade of noradrenergic transmission by either α- or β-antagonists counteracts the stimulatory effect of LHRH on lordosis in ovariectomized estrogen primed rats with or without sexual experience. By contrast, noradrenergic blockers do not interfere with the behavior induced by estrogen only.

LHRH Lordosis Adrenergic receptor Sexual experience

LUTEINIZING hormone-releasing hormone (LHRH) triggers lordosis behavior in ovariectomized estrogen primed rats (2, 8, 19, 20, 22). The cellular mechanisms activated by this peptide to facilitate lordosis have been scarcely explored. One study (9) suggests that the stimulatory action of LHRH on lordosis involves a noradrenergic link, since: a) propranolol, a β-adrenergic blocker, prevents in estrogen primed rats the stimulatory effect on lordosis of intrahypothalamic LHRH when infused along with the peptide, and b) methoxamine, an α-adrenergic agonist that prevents noradrenaline release (30), blocks the action of LHRH in the same preparation. The main objective of the present work was to extend our knowledge on the possible role of the noradrenergic system in the facilitation of lordosis by LHRH. Therefore, we tested the effect of the systemic administration of α- and β-adrenergic antagonists on the lordosis induced by systemic LHRH administration in estrogen primed rats. It was initially found that estrogen primed, sexually inexperienced rats showed inconsistent and weak levels of lordosis after LHRH administration, while repeated testing increased and consolidated LHRH-induced lordosis. We therefore decided to explore the effect of noradrenergic blockers in both sexually inexperienced and in experienced subjects (Ss).

METHOD

Adult Wistar female rats weighing between 220–250 g were bilaterally ovariectomized under ether anesthesia and housed in a controlled light:dark environment (14 hr light:10 hr dark) maintained at 23±2°C. They were fed Purina rat chow and tap water ad lib. All rats received, three weeks after ovariectomy, 5 μg estradiol benzoate (EB; Sigma, St. Louis, MO; SC in 0.1 ml carthamus oil).

Experiment 1

Effect of adrenergic antagonists on the lordosis behavior induced by LHRH in estrogen primed sexually inexperienced rats.

Forty hours after the EB injection, rats were subjected to one of the following treatments:

Group 1:	saline	(n=17)
Group 2:	5 μg LHRH + saline	(n=47)
Group 3:	5 μg LHRH + propranolol 4 mg/kg	(n=22)
Group 4:	5 μg LHRH + propranolol 20 mg/kg	(n=24)
Group 5:	5 μg LHRH + prazosin 0.04 mg/kg	(n=10)
Group 6:	5 μg LHRH + prazosin 0.2 mg/kg	(n=9)
Group 7:	5 μg LHRH + prazosin 1 mg/kg	(n=11)

TABLE 1

EFFECT OF ALPHA- AND BETA-ADRENERGIC ANTAGONISTS ON THE LORDOSIS INDUCED BY LHRH IN OVARIECTOMIZED, ESTROGEN PRIMED, SEXUALLY INEXPERIENCED RATS

Group	N	Treatment	Response at 4 hr		Response at 9 hr	
			LQ	LS	LQ	LS
1	17	Saline	12 ± 19†	23 ± 42*	22 ± 31	39 ± 56
2	47	LHRH + saline	38 ± 41	88 ± 100	31 ± 37	66 ± 86
3	22	LHRH + propranolol 4 mg/kg	25 ± 37	52 ± 83	27 ± 42	59 ± 96
4	24	LHRH + propranolol 20 mg/kg	18 ± 31†	33 ± 64†	31 ± 39	66 ± 80
5	10	LHRH + prazosin 0.04 mg/kg	25 ± 40	50 ± 88	40 ± 41	85 ± 112
6	9	LHRH + prazosin 0.2 mg/kg	8 ± 11*	11 ± 14*	26 ± 32	51 ± 69
7	11	LHRH + prazosin 1.0 mg/kg	5 ± 10	7 ± 15‡	27 ± 27	38 ± 45

Ovariectomized rats received 5 μg EB followed, 40 hr later, by 5 μg LHRH (Groups 2 to 7) or saline (Group 1). Groups 3 to 7 received, along with *and* 2 hr after LHRH, either propranolol or prazosin. Groups 1 and 2 received, instead, saline. Lordosis was tested 4 and 9 hr after LHRH or saline. Data are expressed as means ± S.D. *$p \leq 0.05$; †$p \leq 0.025$; ‡$p \leq 0.01$ compared against Group 2. N=number of subjects in each group.

Adrenergic antagonists (or saline) were given *again* two hours after LHRH. LHRH (Peninsula Laboratories, Belmont, CA) was prepared as a stock solution of 1 mg/ml in acetic acid 0.01 N. Aliquots were frozen and diluted with saline to a concentration of 25 μg/ml immediately before SC injection. Propranolol (Sigma, St. Louis, MO) was dissolved in saline and injected IP in volumes of 0.4–0.7 ml. Prazosin (Pfizer, Groton, CT) was dissolved in 5% glucose-saline, buffered to a pH of about 5.2 with acetic acid 1 N/NaOH 2 N and injected IP in volumes of 0.5–0.8 ml. Tests for lordosis were conducted four and nine hours after LHRH by placing Ss in testing arenas with vigorous males. The lordosis quotient (LQ) and the lordosis score (LS) were determined for each animal as follows:

LQ=mean number of lordosis in 10 mounts × 100
LS=mean lordosis intensity in 10 mounts × 100

Lordosis intensity was rated in a scale of 0 to 3 as suggested by Hardy and De Bold (12). The ability of 5 μg LHRH to induce lordosis in ovariectomized, estrogen primed, sexually inexperienced rats was evaluated by comparing LQ and LS values of Group 2 against those observed in Group 1. The effect of adrenergic antagonists upon LHRH-induced lordosis was assessed by comparing LQ and LS values of groups 3 to 7 against those seen in Group 2. Mann-Whitney U-test (27) was used for all comparisons. Proportions of Ss responding to LHRH in Groups 3 to 7 were compared against those observed in Group 2 by classifying LQs into three categories— low (LQ: 0–30), medium (LQ: 40–60) or high (LQ: 70–100)—and then using a chi square test (27).

Experiment 2

Effect of adrenergic antagonists on the lordosis behavior induced by LHRH in estrogen primed sexually experienced rats.

Forty hours after the EB injection, all rats received a SC injection of 5 μg LHRH and were tested for lordosis as described in Experiment 1 (Test 1). Two weeks later, all Ss received an identical treatment and a second test (Test II). Unresponsive Ss in Test II (about 59% of the population) were discarded from the rest of the experiment. "LHRH-responsive" rats (showing LQs>30) were subjected, two weeks later, to a third treatment and test (Test III), consisting in 5 μg EB and, 40 hours later, one of the following treatments:

Group 8:	saline	(n=10)
Group 9:	5 μg LHRH + saline	(n=8)
Group 10:	5 μg LHRH + propranolol 4 mg/kg	(n=8)
Group 11:	5 μg LHRH + propranolol 20 mg/kg	(n=10)
Group 12:	5 μg LHRH + prazosin 0.2 mg/kg	(n=8)
Group 13:	5 μg LHRH + prazosin 1 mg/kg	(n=8)

Adrenergic antagonists (or saline) were given *again* two hours after LHRH, as in Experiment 1. Solvents, routes of administration, testing procedures and statistical analysis were the same as those described under Experiment 1.

RESULTS

Experiment 1

Administration of 5 μg EB to sexually inexperienced rats induced weak lordosis behavior in 47% of them. However, only two out of the 17 rats in this group showed LQs above 30. Injection of 5 μg LHRH elicited a significant increase in LQ and LS values that peaked at four hours after injection. At nine hours, these values were not significantly different from those observed in rats receiving only EB (see Table 1). The response of sexually inexperienced rats to LHRH was highly variable, 43% of them not responding to all and another 23% displaying intense lordosis (LQs between 70 and 100; see Fig. 1). Propranolol, at the dose of 20 mg/kg, induced a significant reduction in both LQ and LS values four hours after LHRH. Prazosin, at the doses of 0.2 and 1 mg/kg, completely counteracted the facilitatory effect of LHRH. Thus, LQ and LS values observed at four hours postLHRH were even lower than those seen in rats receiving only EB. No rat treated with 0.2 or 1 mg/kg of prazosin showed an LQ above 30. This proportion of Ss was significantly lower ($p=0.05$) than that observed in the control group, where 42% of rats showed LQs above 30. The high dose of

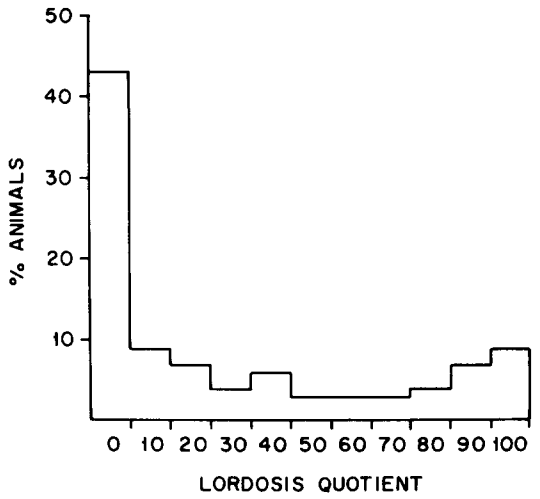

FIG. 1. Distribution of the lordosis quotient observed four hours after the SC administration of 5 μg LHRH to ovariectomized sexually inexperienced rats primed with 5 μg estradiol benzoate 40 hours earlier. n=114.

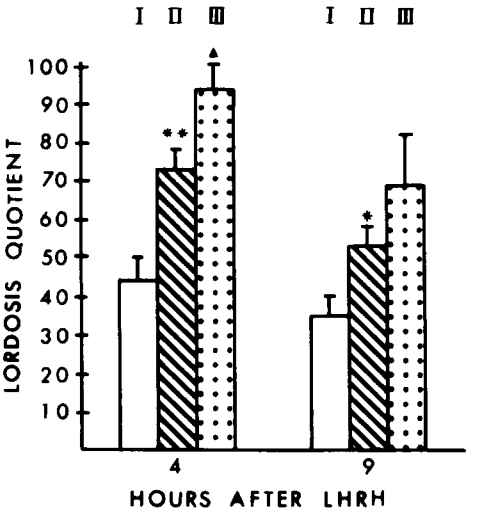

FIG. 2. Increase in the lordosis quotient (LQ) induced by 5 μg LHRH in three successive tests. Ovariectomized rats received 5 μg estradiol benzoate followed, 40 hours later, by 5 μg LHRH, SC. LQ was evaluated 4 and 9 hours after LHRH. This procedure was repeated three times (Tests I, II and III), at two-week intervals. Data are expressed as means±S.E.M. Test I, n=51; Test II, n=51; Test III, n=8 $*p \leq 0.01$, $**p \leq 0.001$, compared against Test I. ▲$p \leq 0.05$ compared against Test II.

prazosin clearly reduced locomotor activity, an effect suggestive of sedation. However, the intermediate dose, which also blocked the effect of LHRH, had no overt effect on locomotion. These results show that blockade of α-adrenergic activity totally prevents the effect of LHRH on lordosis in sexually inexperienced rats, while weaker though significant reductions in lordosis are seen after blocking β-adrenergic activity.

Experiment 2

Figure 2 shows that the responses of estrogen primed rats to LHRH increased significantly from the first to the second test. A more modest, though still significant, increase appeared between the second and the third test. This last increase involves a greater responsivity to both LHRH and to estrogen, since: a) the administration of EB alone on Test III induced significant levels of lordosis in nearly all Ss (see Group 8, Table 2); b) the administration of LHRH to estrogen primed Ss induced, four hours later, levels of lordosis that were significantly greater than those observed in Test II. This finding agrees with previous evidence showing that responsivity to ovarian hormones increases with repeated exposure (1, 10, 13, 23). As shown in Table 2, prazosin—at both dose levels—and propranolol—at the high dose—induced discrete but still significant reductions in lordosis (range of LQ decreases varied between 22% and 37%). Interestingly, these LQs did not significantly differ from those obtained after EB alone, thus showing that in sexually experienced Ss noradrenergic blockers interfered with LHRH-induced lordosis but did not antagonize estrogen-induced lordosis.

DISCUSSION

The present results show that both α- and β-adrenergic antagonists interfere with LHRH-induced lordosis in ovariectomized estrogen primed rats. These data agree with our previous report on the blockade of progesterone-induced lordosis by α- and β-adrenergic antagonists (6). Moreover, they support our proposition that both α- and β-receptors synergize to facilitate lordosis (7), in agreement with the biochemical evidence of Daly and collaborators (4). These investigators found that in several brain areas, including the hypothalamus, the activation of both α- and β-adrenergic receptors is required to obtain increases in cyclic AMP similar to those provoked by noradrenaline (NA). Therefore, responses mediated by this neurotransmitter should be decreased by the administration of either α- or β-adrenergic antagonists, as was seen in the present study.

Lordosis behavior in rodents involves the sequential interaction of a "priming" hormone, which sensitizes some components of the neural substrate for lordosis, and a "triggering" hormone, which releases the behavior and probably regulates its duration (3). While estrogen appears to be the priming hormone, many agents can trigger lordosis in estrogen primed rats (e.g., progesterone, LHRH and prostaglandin E2). We have previously suggested (3) that these chemically heterogenous "triggering" agents stimulate lordosis through their convergence on a common intermediate mechanism. Since both progesterone- and LHRH-induced lordosis are blocked by adrenergic antagonists [(6,9), present study], it would appear that NA acts as the intermediate in the action of these hormones. Two alternatives can be proposed to explain the interaction between NA and LHRH on lordosis: a) LHRH stimulates the release of NA, which in turn activates neurons stimulatory to lordosis, or b) NA synergizes with the action of LHRH on the same neural substrate. While no experimental evidence supports the former alternative, there is some data to support the latter proposition. Thus, cell bodies and fibers containing LHRH have been observed in close proximity to noradrenergic fibers (14–16, 32) in brain areas where the local application of

TABLE 2

EFFECT OF ALPHA- AND BETA-ADRENERGIC ANTAGONISTS ON THE LORDOSIS INDUCED BY LHRH IN OVARIECTOMIZED, ESTROGEN PRIMED, SEXUALLY EXPERIENCED RATS

Group	N	Treatment	Response at 4 hours		Response at 9 hours	
			LQ	LS	LQ	LS
8	10	Saline	51 ± 41†	118 ± 104	48 ± 43	104 ± 100
9	8	LHRH + saline	94 ± 18	186 ± 37	69 ± 38	136 ± 73
10	8	LHRH + propranolol 4 mg/kg	64 ± 46	182 ± 133	78 ± 34	225 ± 103
11	10	LHRH + propranolol 20 mg/kg	74 ± 32*	149 ± 67	62 ± 39	121 ± 81
12	8	LHRH + prazosin 0.2 mg/kg	59 ± 38†	121 ± 83	44 ± 48	97 ± 117
13	8	LHRH + prazosin 1 mg/kg	63 ± 20‡	109 ± 50‡	69 ± 34	131 ± 70

Ovariectomized rats received 5 μg estradiol benzoate (EB) followed, 40 hours later, by 5 μg LHRH. The lordosis quotient (LQ) and lordosis score (LS) were determined 4 and 9 hours after LHRH. This procedure was performed twice at two-week intervals (Tests I and II). Animals showing LQs >30 on Test II were subjected, two weeks later, to Test III. They were given EB plus either saline (Group 8) or LHRH (Groups 9 to 13) as before. Adrenergic antagonists (Groups 10 to 13) or saline (Group 9) were given along with *and* two hours after LHRH. LQ and LS values were determined 4 and 9 hours after LHRH or saline. Data are expressed as means ± S.D. *$p \leq 0.05$, †$p \leq 0.01$, ‡$p \leq 0.002$, compared against Group 9. N=number of animals in each group.

LHRH stimulates lordosis (8, 18, 24–26, 28, 29). Moreover both LHRH and NA trigger in their target cells increases in cyclic AMP (4, 17, 21, 31), thereby providing the cellular basis for additive or synergic effects between them. The participation of a NA link in the hormonal facilitation of lordosis was initially supported by studies showing that lesions of the ventral noradrenergic bundle or chemical depletion of NA interfered with the display of lordosis in rats treated with estrogen and progesterone (5,11). It was suggested that a noradrenergic neuron was a component of the afferent pathway of the lordosis reflex arc (11). However, our finding that noradrenergic blockers, in dosages that completely antagonized the effect of LHRH, failed to interfere with the effect of estrogen in experienced Ss, contradicts this suggestion. Rather, our results support the idea that a noradrenergic link is part of a facilitatory system to the reflex arc for lordosis, which is in turn activated by LHRH and possibly also by progesterone.

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