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Effect of Luteinizing Hormone-Releasing Hormone Antiserum on Sexual Behavior in the Female rat[1]

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COOPER, R. L., M. SEPPALA AND M. LINNOILA. *Effect of lutenizing hormone-releasing hormone antiserum on sexual behavior in the female rat.* PHARMACOL BIOCHEM BEHAV **20**(4) 527–530, 1984.—Various components of sexual receptivity were measured in ovariectomized, estrone-primed female rats following direct placement of luteinizing hormone releasing hormone (LH-RH) or a LH-RH antiserum into the medial preoptic area. Two hours after treatment with LH-RH antiserum, rats showed a significant increase in lordosis behavior indicative of increased sexual receptivity. When tested 3 and 7 hours after treatment, both LH-RH antiserum and LH-RH-treated rats displayed increased lordosis behavior. Similar treatment with a specific peripheral LH-RH agonist and antagonist had no effect on sexual behavior. Proceptive behavior was absent or minimal in all groups and therefore was not affected by the different treatments. Similarly, there was no difference in the rejection quotients of the females representing the various treatment groups. These results demonstrate that the same behavioral response can be observed in animals treated centrally with LH-RH and a highly specific LH-RH antiserum. Similar treatments with a specific peripheral LH-RH agonist or antagonist were without effect. These results suggest that the characteristics of LH-RH recognition sites in the brain are different from those in the pituitary.

Sexual behavior Luteinizing hormone releasing hormone LH-RH antiserum LH-RH agonist LH-RH antagonist

PREVIOUS studies have shown that central administration of LH-RH into the medial preoptic area (MPOA), arcuate nucleus [5,10] and central gray [12,13] elevate lordotic behavior in ovariectomized, estrogen-primed female rats. These findings suggest that this peptide may function as a neurotransmitter or neuromodulatory substance in the brain. LH-RH infused locally into the MPOA or arcuate region results in increased neuronal firing [11]. Furthermore, Beyer *et al.* [1] have provided evidence that LH-RH increases cyclic AMP levels in the neurons related to the expression of lordosis. These observations suggest that the neurons in these regions serve to control and coordinate both the behavioral and endocrine events surrounding reproductive function.

If LH-RH is naturally involved in the expression of lordosis behavior, one would anticipate that blocking the central action of LH-RH would decrease its occurrence. Evidence that such is the case is equivocal. Infusion of LH-RH antiserum into the third ventricle [9] or central gray area [13,14] has been reported to decrease the lordotic response in estrogen-primed female rats, while infusion of LH-RH antiserum into the arcuate region had no effect [4]. Also, systemic or central treatment with certain LH-RH agonists or antagonists does not always modify sexual behavior in the expected manner. Thus, injection of a potent LH-RH antagonist has been shown to induce lordosis in the estrogen-primed female rat [4, 7, 14, 17], whereas similar treatment with a potent LH-RH agonist had no effect or suppressed [7,14] sexual receptivity. Thus, the LH-RH analogs can exert different effects on behavioral and pituitary function.

In preliminary experiments we noted that intraperitoneal injections of LH-RH antiserum, in doses sufficient to block the LH surge in intact animals, did not block sexual behavior in female rats tested during the evening of vaginal proestrus. We also noted that infusion of the antiserum into the third ventricle at two-hour intervals over the day of vaginal proestrus had no effect on the lordosis behavior of intact

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females (i.e., all females were sexually receptive). These observations prompted us to further investigate the relative effect of treatment with an LH-RH antiserum, a specific LH-RH receptor agonist, antagonist and LH-RH itself on the sexual behavior of the female rat by applying the various substances directly to the MPOA.

METHOD

Four-month-old, female Long Evans hooded rats obtained from Charles Rivers (Wilmington, MA) were maintained in an air-conditioned room (22±2°C) within the Duke University's Center for Aging animal facility under a controlled light/dark cycle (lights on between 0500–1900 hr); food and water were available ad lib.

Three weeks prior to behavioral testing, each rat was anesthetized with sodium pentobarbital (Nembutal), 50 mg/kg intraperitoneally, and a stainless steel double barrel cannula, consisting of a 22 g outer tubing (guide cannula) and 27 g inner tubing, was stereotaxically implanted into the medial preoptic area as described previously [2]. Immediately after stereotaxic surgery, each rat was ovariectomized.

Twenty-one days after ovariectomy, the animals were divided into ten groups. The rats in groups 1–8 were injected subcutaneously with estrone (500 μg/kg, dissolved in sesame oil) at 1200 hr. This dose of estrone alone was too low to elicit lordosis behavior when ovariectomized animals were tested with a sexually active male 54–60 hr later. The estrone-primed rats receiving MPOA stimulation with LH-RH (group 1, N=9), LH-RH antiserum (group 2, N=8), LH-RH agonist (group 3, N=12), LH-RH antagonist (group 4, N=8) and lyophilized normal rabbit serum (group 5, N=8) were treated at 1600 hr on Day 23. This was accomplished by removing the inner cannula, tamping a small amount of the crystalline form of the substance (less than 2.0 μg) into its lumen, and immediately returning it into the guide cannula. After treatment the animals were returned to their home cage until behavioral testing. After behavioral testing, the inner cannulae were removed again and the tips inspected for any residue. The lumen of each cannula containing the LH-RH agonist, LH-RH antagonist or LH-RH was clear while the lumen of each cannula containing NRS and LH-RH antiserum remained occluded. The animals in group 6 ("sham" group, N=9) were handled similarly but the cannulae were replaced empty. The estrone-primed animals in groups 7 and 8 (N=8) were given a subcutaneous injection of progesterone (2.5 mg/kg, dissolved in sesame oil) at 1200 hr on Day 23. The estrone-plus-progesterone-treated rats in group 8 (N=8) were subsequently given LH-RH antiserum at 1600 hr as described above. The animals in groups 9 (N=6) and 10 (N=6) were injected with sesame oil on Day 21 and treated with LH-RH or LH-RH antiserum at 1600 hr on Day 23 as described above.

Each female rat was tested with a sexually active male at 1800, 1900 and 2400 hr. Sexual receptivity was expressed as the lordosis quotient (LQ). For each female, at each test time, the LQ was computed by dividing the total number of lordotic responses by the total number of mounts with thrust and multiplying by 100. The rejection quotient (RQ) was also computed for each female on each test day by dividing the total number of rejections (i.e., kicking or turning and fighting) of the male by the total number of mounts or mount attempts [3]. In addition, the occurrence of ear-wiggling and darting (indicative of animal's proceptivity) were also recorded as described previously [3] for each behavioral test. A female was given a score of zero if these behavioral responses were not observed, one if the response was observed once or twice, and two if the response was observed more than twice.

Substances tested in this study included LH-RH, the LH-RH agonist ([D-Ala^6, Des-Gly^{10}]-LH-RH ethyl amide), and the LH-RH antagonist ([D-pGlu^1, D-Phe^2, D-Trp^{3,6}]-LH-RH) which were obtained from Peninsula Laboratories (San Carlos, CA). The LH-RH antiserum was raised in male rabbits by immunizing with synthetic LH-RH coupled to bovine serum albumin [15]. The immunogenic site of LH-RH reacting with the antiserum resides between Tyr^5 and Gly^{10}-NH$_2$ [16]. Other peptides, polypeptide hormones or proteins, including TRH, somatostatin, oxytocin, arginine^8-vasopressin, angiotensin II, a-MSH, insulin, glucagon, pregnancy-specific beta-1 glycoprotein, human chorionic gonadotropin, human placental lactogen and placental protein 5 did not give any cross reaction [16]. Lyophilized normal rabbit serum was obtained from the basal serum of the same animal.

The data were analyzed using a two-way repeated measures analysis of variance (ANOVA) with the treatment serving as the between subjects factor and the time serving as the within subjects factor. Subsequent comparison between groups were made with the Duncan's Multiple Range test. Differences were considered significant when the two-tailed probability values were less than or equal to 0.05.

RESULTS

The mean lordosis quotients for the animals in groups 1–8 are shown in Fig. 1. The repeated measures ANOVA revealed a significant effect of time and treatment and a time × treatment interaction. Sexual receptivity was negligible or absent at 1800, 2000 and 2400 hr in the estrone-primed, sham-treated rats (group 6) and in rats treated with normal rabbit's serum (group 5). In contrast, the mean lordosis quotient of the estrone-primed females treated subsequently with progesterone (group 7) was significantly higher than the mean lordosis quotients of the two control groups when tested at all three times (all $p<0.01$). The mean LQ of the estrone-primed females treated centrally with LH-RH was not different from that of the females treated with estrone only or estrone plus normal rabbit's serum when tested at 1800 hr (mean LQ=0.00). However, the mean LQ of the animals receiving LH-RH was significantly greater than that of the two control groups (groups 5 and 6) at 2000 hr ($p<0.01$) and 2400 hr ($p<0.05$). The mean LQ of the LH-RH-treated animals was not different from the estrone-plus-progesterone-treated rats (group 7) at 2000 hr and 2400 hr. Similarly, the mean LQ of the rats treated with LH-RH antiserum was significantly higher than that observed in the control groups (groups 5 and 6) at 1800 hr ($p<0.01$), 2000 hr ($p<0.001$) and 2400 hr ($p<0.001$). The mean lordosis quotients of the rats in the LH-RH antiserum-treated group was similar to those observed in the estrone-plus-progesterone group at all three test times. LH-RH antibody treatment at 1600 hr did not alter the effect of progesterone (injected at 1200 hr) on lordosis behavior (group 8; Fig. 1). The mean lordosis quotient of the rats treated with sesame oil on Day 21 and LH-RH or LH-RH antiserum on Day 23 (groups 9 and 10) was zero at all three times tested (data not shown).

The mean LQ of the females treated with the LH-RH

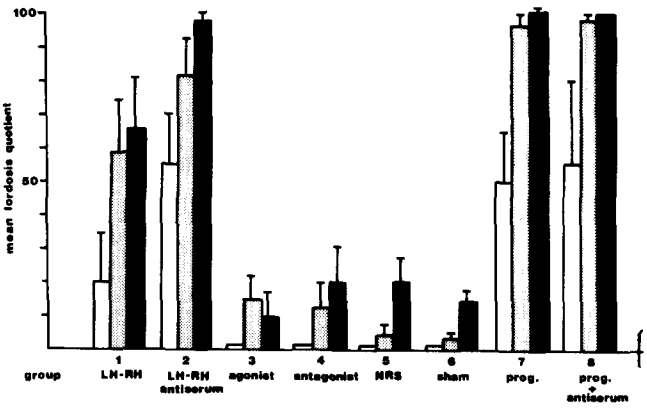

FIG. 1. Mean lordosis quotients (±SEM) of animals assigned to groups 1–8. Open bars=1800 hr, stippled bars=1900 hr and black bars=2400 hr. See text for treatment details.

agonist (group 3) or the LH-RH antagonist (group 4) was not different from the control females (groups 5 and 6) at any time point.

The mean RQ and percent of females that fought or kicked the males in response to his mount attempts did not differ among the groups. The incidence of ear-wiggling and darting was too low for statistical comparison. Ear wiggling and darting was observed in only two estrone-plus-progesterone-treated females and one female receiving the LH-RH antiserum treatment.

DISCUSSION

Our results confirm the findings of Moss and Foreman [10] that the infusion of LH-RH into the MPOA facilitates sexual receptivity in the estrogen-primed female rat. We also found that treatment with LH-RH antiserum in the same area facilitates sexual responsiveness in the estrogen-primed female rat. The efficacy of both treatments depended on the presence of estrogen as similar treatment with the antisera or LH-RH alone was without effect. The finding that LH-RH antiserum placed into the MPOA facilitated lordosis behavior is in contrast with previous reports noting a decrease in receptivity after LH-RH antiserum was injected intracerebroventricularly or into the mesencephalic central gray area [9,13] and no change in behavior when LH-RH antiserum was infused into the arcuate-ventromedial hypothalamic area [4]. The reason for the differential response to LH-RH antiserum reported in the present study and the previous studies may reflect differences in the brain regions tested, strain differences, prior steroid hormone treatment or differences in the binding properties of the antisera used. The possibility that our antiserum could contain other behaviorally active substances, such as progestins, glucocorticoids or ACTH can not be completely discounted at this time. However, the antiserum was highly specific for LH-RH [16]. This antiserum was generated in a male rabbit, and when the pre-immune serum was applied to the MPOA, no change in sexual behavior was noted. It is possible that our LH-RH antiserum stimulated the lordosis response by actually binding to the neuronal LH-RH recognition sites in the brain. It has been demonstrated that a bivalent antibody to insulin receptors can produce a partial insulin-like effect in the liver, perhaps as a result of the antibody binding to insulin recognition sites and causing them to cluster [8]. This clustering, or surface-receptor aggregation, is enough to cause a partial insulin-like effect in this organ. Furthermore, opiate-like effects in neuroblastoma cells have been reported after the application of antisera to opiate-like peptides [6]. Our present finding could represent the behavioral consequences of events similar to those reported to take place in the liver and neuroblastoma cells after exposure to antibodies. If so, this would be the first time that agonistic effects have been described in response to stimulation with a highly specific antibody to a neuropeptide in the brain. Further studies using this strategy are indicated.

The LH-RH analogs used by most investigators have been developed to mimick or block LH-RH effects on LH secretion by the pituitary. The lack of behavioral effects in response to treatment with the LH-RH analogs are in agreement with previous reports. In the present and previous [7,17] studies, treatment with LH-RH agonist did not increase sexual receptivity in females receiving a dose of estrogen that, by itself, was not sufficient to induce sexual behavior. However, Sakuma and Pfaff [14] reported that a LH-RH agonist similar to the one we used ([D-Ala^6, Des-Gly^{10}]-LH-RH ethyl amide) infused into the mesencephalic gray inhibited lordosis behavior in females receiving a dose of estradiol sufficient to induce lordosis behavior in controls. These findings demonstrate that LH-RH analogs have different endocrine and behavioral potencies and suggest that the characteristics of the LH-RH recognition sites within the CNS are different from those in the pituitary. This concept received strong support from the demonstration that some analogs that are antagonistic to LH release can facilitate behavior [4, 7, 14, 17]. The difference between our study and those of Dudley *et al.* [4], Kastin *et al.* [7], Sakuma and Pfaff [14] and Zadina *et al.* [17] in the effects of this type of analog on lordosis may reflect structural differences that are pertinent to the induction of lordosis.

The results of the present study point to the need for a more thorough evaluation of the mechanisms involved in the behavioral effects of treatment with substances that have stimulatory and inhibitory effects on the LH-RH system.

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