Pharmacology Biochemistry & Behavior, Vol. 15, pp. 961–964, 1981. Printed in the U.S.A.

Facilitation of Sexual Receptivity in the Rat by an Ovulation-Inhibiting Analog of LHRH

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Received 28 October 1981

ZADINA, J. E., A. J. KASTIN, L. A. FABRE AND D. H. COY. *Facilitation of sexual receptivity in the rat by an ovulation-inhibiting analog of LHRH.* PHARMAC. BIOCHEM. BEHAV. 15(6) 961–964, 1981.— An LHRH analog known to inhibit ovulation in the rat ([N-Ac-Phe^1, D-p-Cl-Phe^2, D-Trp^{3,6}]-LHRH) was tested for its effects on sexual receptivity. The dose of 500 ng/rat was found in dose-response experiments to be most active and was further investigated for its behavioral effects in rats treated with either estrogen or estrogen plus progesterone. The analog significantly facilitated the behavior of rats in regimens producing low [estradiol benzoate (EB) (2 μg)] and intermediate [EB (2 μg) plus progesterone (2.5 mg)] levels of sexual behavior. In rats given regimens producing high behavioral scores [EB (5 μg) plus progesterone (1 mg)], the peptide did not reduce mating behavior. In the same experiment, rats given EB (5 μg) but not progesterone showed significantly higher scores after the LHRH analog only if they had been designated "responders" by a previous screening test with 1 μg LHRH. These results demonstrate that in animals showing low, intermediate, or high levels of sexual behavior, the LHRH analog can affect mating behavior in a direction quite different from that exerted on pituitary reproductive functions.

Lordosis Receptivity LHRH Mating behavior Analog Antagonist Ovulation

SEXUAL receptivity in the female rat normally begins within 2–3 hours of a preovulatory surge of gonadotropins from the pituitary [2,10], which in turn results from the release of the hypothalamic decapeptide luteinizing hormone-releasing hormone (LHRH) [7,10]. This temporal coincidence, in light of the direct effects of other hypothalamic peptides on the brain [6], led to the discovery in 1973 that LHRH can facilitate lordosis independent of its effects on the pituitary-gonadal axis [8,9]. The concept of independent behavioral and gonadotropin regulatory roles for LHRH was reinforced by the discovery that the ability of synthetic analogs of LHRH to stimulate or inhibit gonadotropin release was not correlated with their receptivity-inducing potency [5]. Particularly striking was the observation that analogs known to inhibit the release of LH were capable of facilitating lordosis. The results of the present study show that one of these analogs facilitated receptivity in animals primed with either low doses of estrogen alone or low doses of estrogen plus high doses of progesterone, a regimen which resulted in intermediate levels of mating behavior. The analog had no inhibitory effect on animals primed with sufficient estrogen and progesterone to show high receptivity.

GENERAL METHOD

Sprague-Dawley derived rats from Zivic Miller weighing about 250 g were obtained 3 days after bilateral ovariectomy, housed in a 12:12 hr L:D room (lights off at 0600), and given Purina Lab Chow and tap water ad lib. Upon arrival, all animals were injected with 5 μg β-estradiol benzoate (EB) (Sigma Chemical Co.) in sesame oil. One week later, they were screened for vigorous copulatory response after a standard injection regimen of estrogen and progesterone. All animals were primed with EB (5 μg at 0 hr) and progesterone (Sigma Chemical Co.) (2.5 mg in sesame oil at 42 hr), and tested at 48 hr. Receptivity was measured by placing the female in a 25×50 cm aquarium that contained a sexually experienced male. The lordosis responses to mounts by the male were recorded. Both the lordosis to mount ratio (L/M) and a receptivity score (RS) were determined, the latter being a rating of 1, 2, or 3 for increasing degrees of arching, a 1 or 2 for increasing duration of holding the lordosis posture, and a 1 if proceptive "darting" occurred. Thus, the maximum possible RS was 6.

One week later, all animals responding (L/M>0.8) to the standard estrogen-progesterone treatment were injected with EB (5 μg at 0 hr), and LHRH (1 μg at 42 hr) and tested for receptivity at 48 hr. Those showing L/M ratios of 0.5 or greater were designated LHRH responders and used in further testing. As described previously [5], this screening procedure reduces variability and facilitates detection of differences in the ability of LHRH analogs to alter lordosis behavior.

All peptides were dissolved in diluent (physiological saline acidified to 0.01 M with acetic acid). Results were analyzed by analysis of variance followed by Duncan's Multiple Range Test.

EXPERIMENT 1: DETERMINATION OF DOSE-RESPONSE PATTERN FOR 2 LHRH ANALOGS

Method

Three weeks after the screening session with LHRH, 2

TABLE 1

	Test	1	2	3	4
Group	1	saline	progesterone	progesterone + LHRH analog 1	LHRH analog 1
	2	progesterone	LHRH analog 1	saline	progesterone + LHRH analog 1
	3	LHRH analog 1	progesterone + LHRH analog 1	progesterone	saline
	4	progesterone + LHRH analog 1	saline	LHRH analog 1	progesterone

analogs previously [5] shown to facilitate lordosis [N-Ac-Phe[1], D-p-Cl-Phe[2], D-Trp[3,6]]-LHRH (analog 1) and [D-pGlu[1], D-Phe[2], D-Trp[3], D-p-NH_2-Phe[6]]-LHRH (analog 2) were tested for activity at several doses (125, 250, 500 and 1000 ng). Initially, groups of 6–7 animals were injected with 2 μg EB followed 42 hr later by one of the analogs (125 ng) or diluent and tested for receptivity at 48 hr. At 5 day intervals, the dose of the analog was doubled and testing was repeated. Two weeks after the test with the 1000 ng dose, a similar series of 4 tests was conducted at 7 day intervals, this time starting at 1000 ng and decreasing the dose by half at each step.

Results

In both series of tests, 500 ng of LHRH analog 1 produced the highest L/M and RS scores followed by analog 2 at 1000 ng. However, only the receptivity scores of animals given 500 ng of analog 1 were significantly ($p<0.05$) greater than controls. This regimen was, therefore, chosen for further experimentation. The control animals also showed slight increases in receptivity over trials in the first series, possibly due to accumulating estrogen or the effects of multiple tests. This tendency was somewhat reduced by using 7 day intervals in the series of descending doses. The means of control animals, however, were not higher than those of the peptide-treated animals in any of the tests.

EXPERIMENT 2: EFFECT OF LHRH ANALOG 1 ON RECEPTIVITY IN ANIMALS PRIMED WITH ESTROGEN OR ESTROGEN PLUS PROGESTERONE

Method

In this experiment, we attempted to determine the effect of LHRH analog 1 in rats primed with estrogen alone or with estrogen plus progesterone. Several weeks after Experiment 1, the 20 animals used in that experiment, together with 12 new females, screened for responsiveness to progesterone and LHRH, as described above, were assigned to 1 of 4 groups. The groups were counter-balanced for previous treatments and randomly assigned to the following conditions: saline, peptide (LHRH analog 1, 500 ng), progesterone (2.5 mg), or progesterone plus peptide. All animals were injected with 2 μg EB at 0 hr, test substance(s) at 42 hr, and tested at 48 hr. At 8 day intervals, each group was given 1 of the other 3 treatments and retested until all animals had received all treatments. The sequence of treatments was balanced for the possible effects of order as shown in Table 1.

TABLE 2

MEAN (±SEM) L/M AND RS AFTER THE VARIOUS TREATMENTS OF EXPERIMENT 2

	L/M	RS
Saline	0.006 ± 0.006	0.006 ± 0.006
LHRH analog 1	0.275 ± 0.069	0.325 ± 0.086
Progesterone	0.331 ± 0.074	0.475 ± 0.134
Progesterone + LHRH analog 1	0.634 ± 0.078	1.063 ± 0.147

Results

In all 4 tests, behavior scores were highest after treatment with the progesterone plus LHRH analog 1 (Table 2). This treatment produced significantly ($p<0.001$) higher L/M and RS scores than any of the other treatments. Injection of progesterone resulted in scores only slightly (NS) higher than those after treatment with peptide, but significantly higher than those after saline for L/M ($p<0.01$) and RS ($p<0.05$). There were no significant differences between the groups, and the groups by treatment interaction was not significant, indicating that the sequence of administration did not significantly affect the results. The overall means for each treatment are shown in Table 2.

EXPERIMENT 3: EFFECTS OF LHRH ANALOG 1 AFTER A PRIMING REGIMEN DESIGNED FOR "HIGH RECEPTIVITY"

Method

A single injection of the near-threshold dose of 2 μg EB was used in earlier experiments to maximize detection of facilitory effects of the LHRH analogs. This resulted in low receptivity scores, even in rats receiving progesterone. In order to test the effects of the antagonist under conditions of higher receptivity, a new set of animals was tested using the

higher estrogen and lower progesterone dosages employed in the "progesterone screening" paradigm described above. The animals were first screened for responsiveness to LHRH as described above, but in order to detect differential responsiveness to the various hormonal combinations by responders and nonresponders, both groups were included in this experiment. 5 μg EB were injected at 0 hr followed by progesterone (1 mg), peptide (500 ng), both substances, or diluent at 42 hr.

Results

As expected, the LHRH screening procedure resulted in the identification of a group of animals showing greater sexual behavior in all treatment conditions (Fig. 1). The main effect of screening was significant $F(1,40)=4.26$, $p<0.05$ for L/M scores. The main effect of hormonal manipulation was significant for both L/M $F(3,40)=9.61$, $p<0.001$ and RS $F(3,40)=8.48$, $p<0.001$. Animals given progesterone, with or without peptide, showed significantly higher L/M scores than both diluent groups ($p<0.02$) as well as nonresponders given peptide ($p<0.05$). Scores for responders given LHRH analog 1 alone were also significantly higher than those of both diluent groups ($p<0.02$) and those of similarly treated nonresponders ($p<0.05$). The peptide did not significantly affect the scores of progesterone-treated animals. RS values showed a similar pattern except that the tendency ($p<0.1$) for peptide-treated responders to show higher receptivity than control groups was not significant.

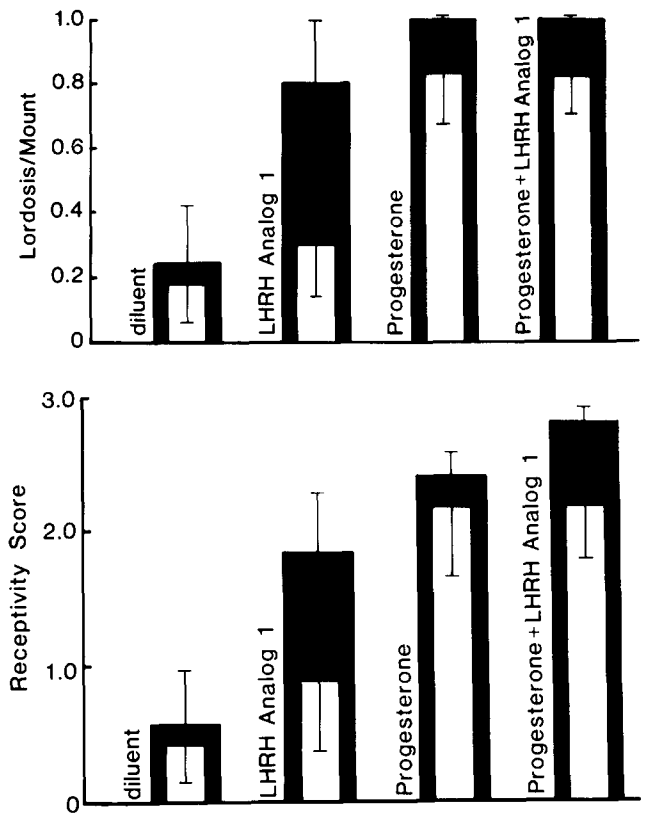

FIG. 1. Lordosis/mount ratio (top) and receptivity score (bottom) for responders (filled bars) and nonresponders (open bars) given diluent, LHRH analog 1, progesterone, or progesterone + LHRH analog 1 in Experiment 3. Values are means ± SEM.

DISCUSSION

In this series of studies, an LHRH analog known to be a potent inhibitor of ovulation facilitated sexual receptivity in rats given doses of estrogen and progesterone that produced low or intermediate levels of mating behavior, and did not inhibit receptivity in rats primed for high levels of sexual behavior. These experiments thus confirm and extend our earlier [5] study in which a regimen of estrogen inducing low receptivity was used. In addition, dose-response results indicated that 500 ng was an optimal dose of this analog for facilitation of sexual behavior in this system.

Dudley *et al.* [3] have recently shown that an analog of LHRH that blocked ovulation also reduced L/M scores in rats given estrone and the dose of progesterone (2.5 mg) used in Experiment 2 of the present study. Our use of 2 μg EB with this dose of progesterone resulted in only intermediate levels of receptivity; these were significantly facilitated by analog 1. Since the estrone-progesterone combination produced high levels of receptivity, we used a regimen of estrogen and progesterone in Experiment 3 that also produced high receptivity. However, no inhibition of sexual activity was seen after injection of LHRH analog 1.

In addition to the differences in the structure of the analog and priming regimen used, other differences between our study and that of Dudley *et al.*, particularly the route of administration (subcutaneous vs intraventricular injection), could also have contributed to the different behavioral responses to ovulation-blocking LHRH analogs in the two studies.

The use of the LHRH screening procedure we proposed previously [5] identified a more responsive subpopulation of animals. The degree to which the increased responsiveness of these animals was due to estrogen, the peptide, or the combination was not discernible in this paradigm. However, as shown in Experiment 3 and previous studies [5], differences between peptide-injected and control groups were more reliably observed with responders. Neither responders nor nonresponders showed any inhibition of receptivity after estrogen and progesterone.

The dose range of the analog used in these experiments was chosen to approximate that at which the parent compound is known to affect lordosis [8,9]. However, in preliminary studies using the larger dose found effective in blocking ovulation (31 μg/rat) [4], LHRH analog 1 slightly facilitated lordosis in EB (5 μg) treated rats and did not inhibit it in animals given EB (5 μg) plus progesterone (1 mg).

This set of studies, in which a competitive antagonist to LHRH at the pituitary level facilitated reproductive behavior, provides another example of the concept [6] that the actions of a peptide on the pituitary can be substantially different from its actions on other systems.

ACKNOWLEDGEMENTS

The authors thank M. Reardon for preparing the manuscript, N. Kunen for the artwork and M. Grove for the photography.

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