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Dissociation of Effects of LH-RH Analogs on Pituitary Regulation and Reproductive Behavior

ABBA J. KASTIN,[1] DAVID H. COY, ANDREW V. SCHALLY AND JAMES E. ZADINA

Veterans Administration Medical Center and
Tulane University School of Medicine, New Orleans, LA 70146

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KASTIN, A. J., D. H. COY, A. V. SCHALLY AND J. E. ZADINA. *Dissociation of effects of LH-RH analogs on pituitary regulation and reproductive behavior.* PHARMAC. BIOCHEM. BEHAV. 13(6) 913–914, 1980.—Analogs of LH-RH were studied for their effect on lordosis in ovariectomized rats primed with estrogen. Classified by their gonadotropin-releasing activity, representatives of three types of analogs were tested for facilitation of lordotic (L) responses of preselected females to mounting (M) by male studs. Positive responses (L/M>0.5) were found after SC administration of LH-RH peptides modified so as to be inhibitory, stimulatory, or inactive in releasing LH and FSH. The results further support the concept of a dissociation between the endocrine and extra-endocrine effects of peripherally injected hypothalamic peptides.

Mating Lordosis Behavior Peptides LH-RH Pituitary Hypothalamus

IN 1973, our concept of the "extra-pituitary" or "extra-endocrine" actions of hypothalamic peptides on the central nervous system (CNS) [4] was extended to the decapeptide luteinizing hormone-releasing hormone (LH-RH). Two groups of investigators [5,7] showed that increased lordosis caused in female rats by injection of LH-RH was not dependent on alteration of pituitary or gonadal hormonal levels. This provided another example of the dissociation between actions exerted by hypothalamic peptides on the brain and actions exerted on the pituitary.

The present study reports the effects on female rats of three different types of synthetic analogs of LH-RH. It emphasizes the dissociation between pituitary and extra-pituitary effects since the action of these analogs on mating behavior did not correlate with their action on releasing the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland.

METHOD

Ovariectomized rats weighing about 250 g were obtained from Zivic Miller Co. (Allison Park, PA) three days after surgery at which time they received the first injection of β-estradiol benzoate (Sigma Chemical Co.) in sesame oil (5 μg/rat SC). One week later, the administration of estrogen was repeated. A day or two later, "screening" of the females began with the injection of 5 μg estradiol benzoate followed in two days by LH-RH (1 μg/rat SC). All rats were housed in a reversed light-dark cycle with free access to food and water. Testing began 6 hours after LH-RH injection, 2.5 hours into the dark cycle. Two vigorous males were placed in the observation arena for 10 minutes before the female was introduced. Mating behavior was observed for 5 minutes, during which time a mean of 6.5 mounts occurred. The males were usually changed after four females. Those female rats showing a lordosis to mount (L/M) ratio greater than 0.5 were designated "responders" to LH-RH and used in the study.

A few days later, "responders" to LH-RH received 5 μg estradiol benzoate followed in two days by one of the following: 1 mg progesterone (Prolutin, Schering Corp.) in sesame oil, SC (active control), diluent (inactive control), or peptide (500 ng SC). Mating tests were conducted as described above.

All peptides were synthesized by solid-phase methods and highly purified. They were put into a solution of 0.01 M acetic acid in 0.9% NaCl (diluent) immediately before use. Some peptides were initially dissolved in propylene glycol (<30%). The 8 analogs used in this study fall into three categories. The first category consists of antagonists, known to inhibit gonadotropin release (N-Ac-Phe[1],D-p-Cl-Phe[2],D-Trp[3,6]-LH-RH, N-Ac-D-Phe[1],D-p-Cl-Phe[2],D-Trp[3,6], D-Ala[10]-LH-RH, D-pGlu[1],D-Phe[2],D-Trp[3],D-p-NH_2-Phe[6]-LH-RH, D-pGlu[1],D-Phe[2],D-Trp[3],D-Phe[6]-LH-RH); the second group contains superactive LH-RH analogs, known to be more active than the parent LH-RH in stimulating gonadotropin release

[1]Send reprint requests to Dr. Abba J. Kastin, Endocrinology Sect. of the Medical Service, Veterans Administration Medical Center, 1601 Perdido Street, New Orleans, LA 70146.

from the pituitary gland (D-Phe^6,desGly^{10}-LH-RH-propylamide, D-Leu^6, desGly^{10}-LH-RH-ethylamide); the third group consists of analogs of LH-RH which are inactive in either stimulating or inhibiting the release of gonadotropins (D-Trp^3-LH-RH, D-Tyr^5-LH-RH) ([1, 2, 8] and unpublished observations).

RESULTS AND DISCUSSION

As expected, estrogen-primed female rats given progesterone showed high mean L/M ratios, usually close to 1.0, while those given diluent seldom showed any lordosis. At least one analog from each category (inhibitory, inactive, and superactive with respect to gonadotropin release) facilitated lordosis; the potent antagonist (D-pGlu^1, D-Phe^2, D-Trp^3, D-p-NH_2-Phe^6-LH-RH), the inactive analog (D-Trp^3-LH-RH) and the superactive analog (D-Phe^6,desGly^{10}-LH-RH-propylamide) produced mean L/M ratios between 0.50 and 0.60.

Since the inhibitory analogs seemed to most dramatically illustrate the dissociation between endocrine and extra-endocrine effects, two additional antagonists were tested. These two compounds (N-Ac-Phe^1,D-p-Cl-Phe^2,D-Trp^{3,6}-LH-RH), (N-Ac-D-Phe^1,D-p-Cl-Phe^2,D-Trp^{3,6},D-Ala^{10}-LH-RH) have considerably higher inhibitory activity with respect to gonadotropin release than the other antagonist tested (D-p-Glu^1,D-Phe^2,D-Trp^3,D-p-NH_2-Phe^6-LH-RH), but all three facilitated lordosis. All of these lordosis-active analogs seemed to give more consistent stimulation than did the parent LH-RH at 500 ng.

Initially, we experienced difficulty in obtaining reliable increases in lordotic behavior. Preselection of responders by previous administration of 1 μg LH-RH appeared to greatly reduce variability and facilitate detection of potentiating activity. In a direct test of this screening procedure technique, an active LH-RH antagonist was injected into 12 ovariectomized rats, half of which had been shown on the previous day to respond to LH-RH and half of which did not. No lordosis after injection of the inhibitory analog of LH-RH was observed in any of the six non-responders in contrast to a mean L/M ratio of 0.91 in the responders.

At an earlier stage of the study, before the technique of preselection of females was devised, we observed the first indications of activity for some of the same compounds mentioned above. During this time, we also found representatives from all three types of analogs which were apparently inactive in facilitating lordotic behavior. These peptides included an antagonist of gonadotropin release (D-pGlu^1, D-Phe^2,D-Trp^3,D-Phe^6-LH-RH), an inactive analog (D-Try^5-LH-RH), and a superactive releaser of gonadotropins (D-Leu^6,desGly^{10}-LH-RH-ethylamide). However, there was more variability with this preliminary method of testing in which only 2–5 unselected female rats were used for each peptide as compared with the 6–9 responders used for each peptide in the later experiments. Although the subsequent tests with prescreened females focused on analogs showing indications of activity, these earlier results provide preliminary indications that analogs representing each of the three gonadotropin-regulatory categories may be inactive in changing lordosis behavior. Taken together with the above observations on lordosis-active analogs from all three categories, the results support the concept of a dissociation between pituitary and extra-pituitary effects of peptides.

The clinical studies of LH-RH on sexual behavior in human beings have been recently reviewed [3,6]. There has been a paucity of positive findings. Among the possible explanations for the apparent discrepancy between the studies in human beings and those in rats are inherent species differences, predominant use of females in the animal studies and males in the human studies, dosage, less variability in relatively inbred rats, more complex psychological factors in human beings, and the use of only the parent LH-RH in the clinical trials. Regardless of any potential clinical applicability, the studies with analogs of LH-RH in rats reinforce the existence of extra-pituitary, extra-endocrine effects of hypothalamic peptides after peripheral administration.

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