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Ovarian Growth and Uptake of Iodinated D-Leu^6, des Gly $NH_2{}^{10}$-LHRH Ethylamide in HCG Treated Rats [1, 2] (40523)

MOHAMMAD Q. MAYAR,[3] GLADYS K. TARNAVSKY, AND JERRY J. REEVES

Department of Animal Sciences, Washington State University, Pullman, Washington 99164

A number of biologically potent stimulatory analogs to luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LHRH) have been synthesized. After a subcutaneous injection of the analog D-Leu^6, des Gly $NH_2{}^{10}$-LHRH-ethylamide (D-Leu^6-LHRH-EA) into immature male rats, 53 times as much luteinizing hormone (LH) and 15 times as much follicle stimulating hormone (FSH) were released over a 6 hr period as compared to a similar dose of natural LHRH (1). The ovulatory activity of this analog in rats was 70–80 times greater than that of LHRH (2). In spite of the higher biological activity resulting in greater release of LH and FSH, treatments of D-Leu^6-LHRH-EA have not always promoted significant ovarian growth. In fact, Rippel and Johnson (3) reported that large doses of potent D-Leu^6-LHRH-EA inhibited human chorionic gonadotropin (HCG) augmentation of ovarian growth in both intact and hypophysectomized prepubertal rats. This apparent extrapituitary inhibition by the LHRH analog has lead to the design of the present study which was to determine if D-Leu^6-LHRH-EA acts directly on the ovary rather than indirectly through the pituitary hormones. Tissue and pituitary uptake of ^{125}I-D-Leu^6-LHRH-EA, ovarian growth and serum LH and FSH concentrations were evaluated in rats treated with HCG.

Materials and methods. Experiment I. Twenty-one day-old Sprague-Dawley female rats were randomly divided into 4 groups of six rats each and anesthetized with 25% w/v urethane. Rats were injected intravenously (iv) with either 0.3 ml of 0.9% saline (Group I), 50 IU HCG (Group II), 5 μg of D-Leu^6-LHRH-EA (Group III), or 5 μg of D-Leu^6-LHRH-EA plus 50 IU HCG. (Group IV). One min after treatment, all rats were injected iv with 10 μCi of biologically active monoiodinated ^{125}I-D-Leu^6-LHRH-EA. Rats were exsanguinated 30 min after the iodinated analog injection. Ovaries, pituitary, uteri, adrenal, liver, kidney, heart, central nervous system cortex (CNS) and thyroid were collected, weighed and counted for radioactivity immediately after collection. A blood sample was collected prior to treatment and at the time of exsanguination. Serum radioactivity was determined in all posttreatment samples. A ratio of tissue cpm/mg to serum cpm/μl (T/S ratio) was used to express specific radioactive uptake by individual organs. The procedure described by Reeves *et al.* (4) was used to obtain biologically active monoiodinated D-Leu^6-LHRH-EA.

Serum LH concentrations of each blood sample were determined by the method of Niswender *et al.* (5) and expressed in ng/ml NIH-LH-S17. Serum FSH concentrations were determined by NIAMDD kit and expressed as μg/ml NIAMDD rat FSH-RP1. Changes in serum LH and FSH concentrations were obtained by the difference in hormone level before and 30 min after ^{125}I-D-Leu^6-LHRH-EA injection and expressed as ΔLH and ΔFSH.

Experiment II. Thirty-two 21-day-old Sprague-Dawley female rats were randomly divided into 4 equal groups. Rats in each group were injected intraperitoneally (ip) once each day for 3 days with the following doses: Group I rats received 0.3 ml of 0.9% saline, Group II rats received 50 IU HCG, Group III rats received 5 μg of D-Leu^6-LHRH-EA, and Group IV rats received 5 μg of D-Leu^{6}LHRH-EA plus 50 IU HCG. On

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[3] Present address: Faculty of Agriculture, Kabul University, Kabul Afghanistan.

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the third day of treatment, rats were anesthetized with urethane and an iv injection of each treatment was followed one min later with 10 μCi of ^{125}I-D-Leu^6-LHRH-EA. Rats were exanguinated 30 min later, blood and tissues were collected as described with Experiment I.

Experiment III. Twenty 42-day-old female hypophysectomized rats were randomly divided into 4 equal groups. Rats in each group were injected ip once a day for 3 days as described in Experiment II. On the third day of treatment, rats were anesthetized with urethane and an iv injection of each treatment was followed one min later with 10 μCi of ^{125}I-D-Leu^6-LHRH-EA. Rats were exsanguinated 30 min later, blood and tissues except the pituitary were collected as described in the first two experiments. Hypophysectomy was confirmed by microscopic examination of the sella turcia. Statistical comparisons among treatment means for all experiments were made using Tukey's omega-procedure (6).

Results. Experiment I. Uptake of radioactivity, (T/S ratio) measured in uteri, ovaries, pituitary and adrenals after a single injection of saline, HCG, or D-Leu^6-LHRH-EA is shown in Fig. 1a. Pituitaries and ovaries of D-Leu^6-LHRH-EA or D-Leu^6-LHRH-EA + HCG treated rats had lower ($P < 0.05$) T/S ratios than those receiving HCG or saline. There were no significant differences ($P > 0.05$) between the treatment groups for T/S ratios of uteri, adrenal, liver, kidney, heart, CNS cortex or thyroid (Fig. 1b). The radioactive T/S ratio of pituitaries from rats treated with HCG was higher ($P < 0.05$) than the T/S ratio of pituitaries from saline treated rats. Serum LH was lower in HCG treated than saline treated rats after an injection of ^{125}I-D-Leu^6-LHRH-EA ($P < 0.05$) (Table I). Serum LH was released in saline pretreated group because of the biological actions of labeled D-Leu^6-LHRH-EA. Serum FSH concentrations were not influenced by treatment.

Experiment II. Figure 2 illustrates the T/S ratios of tissues from rats treated daily for 3 days prior to injection of ^{125}I-D-Leu^6-LHRH-EA. The T/S ratio of ovarian and pituitary samples from rats receiving D-Leu^6-LHRH-EA or D-Leu^6-LHRH-EA + HCG were lower ($P < 0.05$) than those treated with HCG or

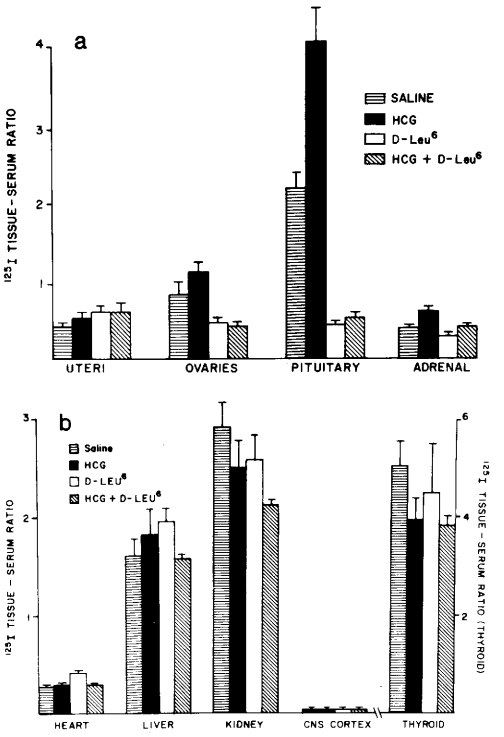

FIG. 1a. Tissue/serum ratio (T/S) of ^{125}I uptake by uteri, ovaries, pituitary and adrenal of rats after acute treatment (Experiment I) with saline, HCG, D-Leu^6-LHRH-EA or HCG plus D-Leu^6-LHRH-EA ($n = 6$).

FIG. 1b. Tissue/serum ratio (T/S) of ^{125}I uptake by liver, kidney, heart, thyroid and CNS cortex after acute treatment (Experiment I) with saline, HCG, D-Leu^6-LHRH-EA or HCG plus D-Leu^6-LHRH-EA.

TABLE I. SERUM LH AND FSH IN RESPONSE TO LABELED D-LEU^6-LHRH-EA IN RATS PRETREATED WITH D-Leu^6-LHRH-EA AND HCG.

Group treatments	Serum ΔLH[a] (ng/ml)	Serum ΔFSH[b] (μg/ml)
Experiment I		
I Saline	12.0 ± 1.6	4.0 ± 0.7
II HCG	4.7 ± 1.7[c]	3.6 ± 0.7
III D-LEU^6-LHRH-EA	18.9 ± 4.5	4.1 ± 0.7
IV HCG + D-Leu^6-LHRH-EA	20.5 ± 2.3	6.0 ± 0.5
Experiment II		
I Saline	15.1 ± 1.4	2.9 ± 0.8
II HCG	7.3 ± 1.2[c]	3.3 ± 0.9
III D-Leu^6-LHRH-EA	8.7 ± 0.4[c]	2.9 ± 0.9
IV HCG + D-Leu^6-LHRH-EA	6.8 ± 0.4[c]	3.9 ± 1.0

[a, b] ΔLH and ΔFSH are obtained by the difference before and 30 min after injections of approximately 10 ng ^{125}I-D-Leu^6-LHRH-EA.

[c] $P < 0.05$ different than saline treated animals.

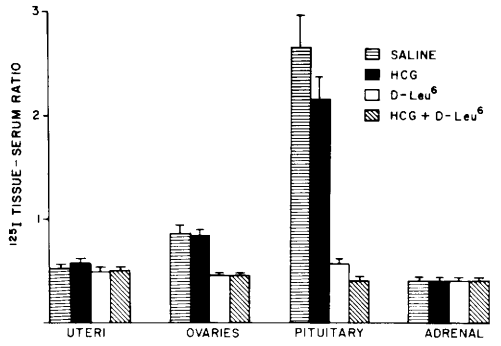

FIG. 2. Tissue/serum ratio (T/S) of ^{125}I uptake by uteri, ovary, pituitary and adrenal of rats after chronic treatment (Experiment II) with saline, HCG, D-Leu^6-LHRH-EA or HCG plus D-Leu^6-LHRH-EA (n = 8).

saline. There were no significant differences in T/S ratios among other tissues. The serum LH values were lower ($P < 0.05$) from rats in Group II, III, and IV compared to serum LH in Group I rats (Table I). Serum FSH values were not different between groups.

Ovarian weights after the 3-day treatment period are shown in Fig. 3. Mean ovarian weight for ovaries of saline and HCG treated rats was 8 mg and 25 mg, respectively. Ovarian weight of D-Leu^6-LHRH-EA treated rats was not significantly ($P > 0.05$) different from that of the control group. HCG in the presence of D-Leu^6-LHRH-EA did not cause significant ($P > 0.05$) ovarian growth.

Experiment III. Mean ovarian weight of the group II hypophysectomized (HCG treated) rats was greater ($P < 0.05$) than ovarian weight for either group I, II or IV rats (Fig. 4). D-Leu^6-LHRH-EA treatment in Group IV rats inhibited the HCG induced ovarian growth. Uptake of ^{125}I as noted by T/S ratio in ovaries was highest in saline-treated rats compared with ovaries from rats treated with unlabeled D-Leu^6-LHRH-EA (Fig. 5). No significant changes in binding ratios were noted in any other tissues assayed.

Discussion. The significantly lower uptake of radioactivity after an acute or chronic treatment with the unlabeled analog alone or in combination with HCG indicates that the analog is competitively taken up by both the pituitary and ovary. This competitive uptake of ^{125}I-D-Leu^6-LHRH-EA by the ovary in the absence of the pituitary might lead to the assumption that the ovary as well as the pituitary have receptors for the analog and

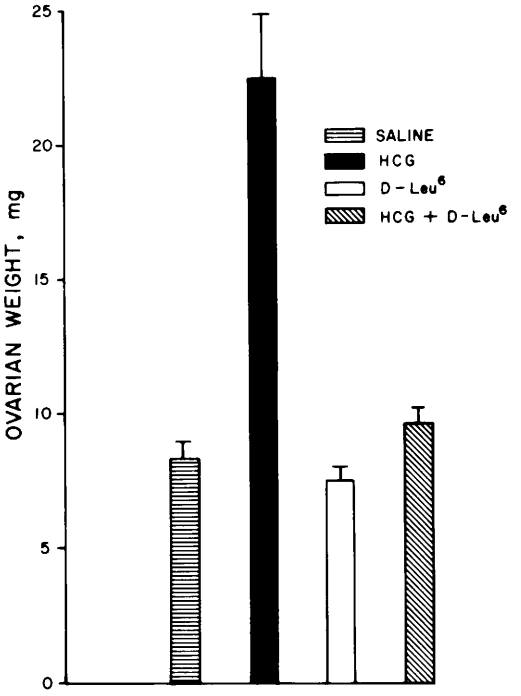

FIG. 3. Ovarian weight of rats after a chronic treatment (Experiment II) with saline, HCG, D-Leu^6-LHRH-EA or HCG plus D-Leu^6-LHRH-EA (n = 8).

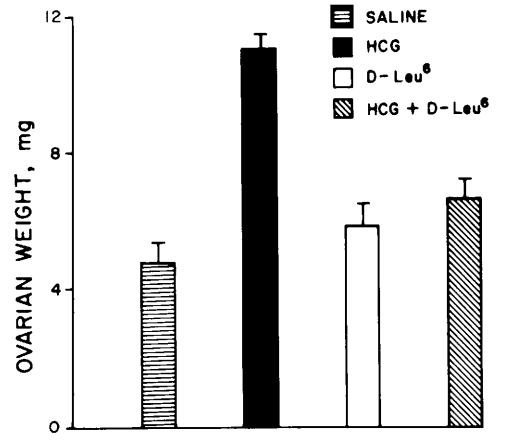

FIG. 4. Ovarian weight of hypophysectomized rats after a chronic treatment with saline, HCG, D-Leu^6-LHRH-EA or HCG plus D-Leu^6-LHRH-EA.

that the LHRH analog acts directly on the ovary to inhibit HCG induced growth. Acute pretreatment with HCG augmented the uptake of ^{125}I-D-Leu^6-LHRH-EA by the pituitary. This was not observed after chronic treatment with HCG. Since HCG does not displace labeled D-Leu^6-LHRH-EA it can be assumed that HCG and D-Leu^6-LHRH-EA

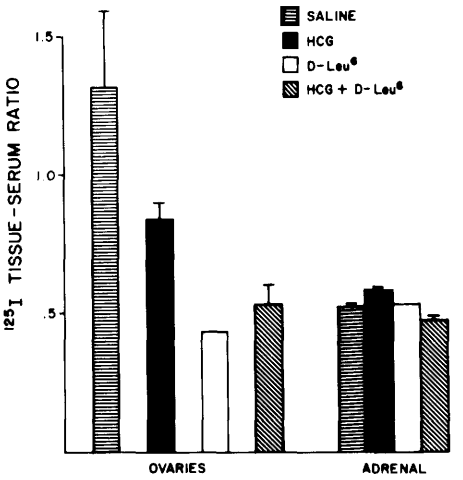

FIG. 5. Tissue/serum ratio (T/S) of ^{125}I uptake by ovary and adrenal of hypophysectomized rats after treatment with saline, HCG, D-Leu^6-LHRH-EA or D-Leu^6-LHRH-EA + HCG.

do not compete for the same receptor site. However, in both experiments there was a decrease in serum LH secretion of HCG treated rats.

In the present study, the inhibitory action of the analog on HCG -induced growth of the ovary confirms the findings of Rippel and Johnson (3). Johnson *et al.* (7) also reported normal ovarian growth inhibition in rats chronically treated with D-Leu^6-LHRH-EA. Strong antifertility effects of a similar LHRH analog D-Ala^6, des Gly $NH_2{}^{10}$-LHRH-ethylamide has also been reported (8, 9). De Sombre *et al.* (10) found that treatment with D-Leu^6-LHRH-EA in rats was essentially as effective as ovariectomy in causing mammary tumor regression. Oshima *et al.* (11) reported that chronic treatment with synthetic LHRH decreased accessory sexual organ weight in the immature male rat. Testicular growth inhibition has also been reported (12) during a chronic D-Leu^6-LHRH-EA treatment. The blockage of testosterone synthesis during LHRH or D-Leu^6-LHRH-EA treatment has also been reported (11, 12). These latter investigators assumed, however, that the mechanism of action was mediated via the pituitary.

The present study lends supportive evidence that the extrapituitary site of action of D-Leu^6-LHRH-EA in inhibiting HCG induced ovarian growth is at least partly at the ovary. The physiological significance of these findings are difficult to evaluate because of the large dose of D-Leu^6-LHRH-EA used in this study. However, these data suggest that there are sites present at the ovary that bind this synthetic analog of LHRH, which may be part of a feedback control mechanism in preventing the ovary from overstimulation by gonadotropins.

Summary. Competitive uptake of labeled and unlabeled D-Leu^6-LHRH-EA was observed in both the pituitary and ovary. HCG induced ovarian growth was inhibited by treating intact and hypophysectomized rats simultaneously with D-Leu^6-LHRH-EA therefore, suggesting that D-Leu^6-LHRH-EA acts directly on the ovary to inhibit HCG induced ovarian growth.

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