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REDUCTION OF LH-RH PITUITARY AND ESTRADIOL UTERINE BINDING SITES BY A SUPERACTIVE ANALOG OF LUTEINIZING HORMONE-RELEASING HORMONE

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SUMMARY

The ability of the Luteinizing Hormone-Releasing Hormone (LH-RH) analogs to displace LH-RH from its pituitary receptors was evaluated in vitro. The two superactive analogs tested showed higher potency than the antagonists and LH-RH itself, D-Trp^6-LH-RH being the most potent. The LH-RH specific binding activity in the pituitary fluctuated throughout the age of the rats. The highest number of LH-RH binding sites were seen on day 35 of age (276 fmol x 10^{-2}/pit) and an increment was induced by 0.05 µg D-Trp^6-LH-RH (400 fmol x 10^{-2}/pit). However, 1 µg D-Trp^6-LH-RH reduced the binding of LH-RH at all the times studied. In the control animals the number of estradiol binding sites increased on day 42 of age, and 0.05 µg D-Trp^6-LH-RH augmented them on day 35 of age. On the contrary, 1 µg D-Trp^6-LH-RH diminished the estradiol uterine receptors at all the times studied. Similar results were obtained in the ovariectomized-hypophysectomized rats on day 35 of age. Our studies demonstrated a biphasic action of D-Trp^6-LH-RH on LH-RH pituitary receptors and a direct effect on uterus which could be mediated through the uterine estradiol receptors.

The incorporation of D-tryptophan in place of glycine in position 6 of the LH-RH decapeptide produces an analog which has far greater gonadotropin-releasing activities in vivo and in vitro than the natural hormone (1). We have observed that this compound, D-Trp^6-LH-RH, prevented implantation in rats when given from days 1 to 5 of pregnancy and significantly reduced progesterone levels in blood (2). In immature female rats it decreased uterine weight, elevated serum gonadotropins, decreased the pituitary LH-content and also reduced the pituitary responsiveness to exogenous LH-RH (3). There is evidence that some of these effects of the superactive analogs could be exerted directly on those extrapituitary tissues (4). We have also shown that D-Trp^6-LH-RH reduced the in vitro binding activity of iodinated LH-RH to its pituitary receptors (5). In this paper, we analyzed

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the effect of several superactive and inhibitory analogs of LH-RH on the *in vitro* binding activity of the parent hormone to its pituitary receptors. On the basis of these results, we have selected the compound which displayed the highest binding competition activity for studying the effects on LH-RH pituitary receptors and estradiol uterine binding sites.

MATERIALS AND METHODS

Animals. Adult or immature female rats of the CD strain (Charles River) were housed in animal quarters which were equipped with controlled lighting (light 0500 hrs to 1900 hrs) and controlled temperature (24 ± 2° C). Animals were fed with Purina chow rat diets and water ad libitum. Adult rats were ovariectomized (OVX) 2 weeks prior to the experiment and the immature animals were hypophysectomized through the auditory canal under Nembutal anesthesia supplemented with ether.

Experiment One: OVX animals were injected subcutaneously with 2 µg 17ß-estradiol dissolved in 5% ethanol in saline and sacrificed by decapitation 24 hrs thereafter. The pituitaries were removed immediately and placed in cold Hepes buffer containing 0.5 mg/ml Bacitracin. The LH-RH and analog-binding studies were done in pituitary homogenates as described previously (5) with the modification that the concentration of cold hormone was reduced to 2.5 and 5 nM. Each tube contained the amount of tissue equivalent to one pituitary and each point was done in triplicate. The LH-RH analogs tested are listed in Table I.

Experiment Two: Immature rats received s.c. injections every day at 0900 hrs from day 30 to day 40 of age of 0.05 µg or 1 µg [D-Trp^6]-LH-RH, dissolved in 0.2 ml 0.9% saline solution. Control rats received injections of vehicle alone. Six animals per group were ovariectomized and hypophysectomized on day 30 and 31 of age, respectively. At 1600 hrs on different days after beginning the experiment, six animals from each group were killed by decapitation and pituitary and uterus were removed and blood collected from the trunk. Serum estradiol radioimmunoassay, LH-RH and pituitary receptors and binding sites for estradiol in uterus were determined as described previously (3,5,6). These parameters were evaluated only on day 35 of age in the ovariectomized hypophysectomized rats.

RESULTS

Ability of the analogs to displace LH-RH from its pituitary receptors.

Table I shows the bioactivity of several LH-RH analogs as well as their potency in the receptor assay. The biological activities of the analogs were taken from our previous studies (1,7-9) in order to compare them with their *in vitro* capacity to displace monoiodinated LH-RH from pituitary receptors. To find the potency of each analog, the LH-RH activity in the same receptor assay was taken as I. The two superactive analogs tested showed higher potency than the antagonists and LH-RH itself, D-Trp^6-LH-RH

TABLE I

Analog	Bio-Activity	Potency in the receptor assay**
Superactive	Releasing LH-FSH *	
[D-Phe^6-des-GlyNH$_2^{10}$]-LH-RH-ethylamide (1)[2]	8-7	15
[D-Trp^6]-LH-RH (1)	13-21	20
Inhibitory	Anti-LH (%)+	
[D-Phe^2,D-Trp^3,D-Phe^6]-LH-RH (7)	95	5
[D-Phe^3,Phe^3,D-Phe^6]-LH-RH (7)	92	1
[D-Phe^2]-LH-RH (8)	30.4	< 1
[desHis^2,desGlyNH$_2^{10}$]-LH-RH ethylamide (8)[2]	40.6	< 1
[D-Phe^2,D-Pro^3,D-Phe^6]-LH-RH (9)	--[0]	< 1

* The activities of the analogs were evaluated in the ovariectomized rat assay and compared with LH-RH [(Pyro)Glu,His,Trp,Ser,Tyr,Gly, Leu,Arg,Pro,Gly-NH$_2$](1).

\+ Anti-LH releasing activities were calculated in terms of the neutralizing effect on LH-RH, comparing the pituitary response to LH-RH with and without analog (7,8).

0 The ability to block ovulation was evaluated previously (9). (1 mg did not block ovulation).

** The potencies in the receptor assay were found by using the same molar concentrations of LH-RH and the analogs (5 nM) to displace a fixed amount of monoiodinated LH-RH (23.4 pM) from its pituitary receptor sites. The activity of LH-RH in the assay was considered 1 and then each analog was compared to this activity.

being the most potent analog. The most active antagonists, [D-Phe^2,D-Trp^3, D-Phe^6]-LH-RH and [D-Phe^2,D-Phe^3,D-Phe^6]-LH-RH (7-9) had potencies higher and similar to LH-RH, respectively.

Effect of D-Trp^6-LH-RH on LH-RH Pituitary Receptors. This was studied in immature female rats at different days of age (Fig. 1). The greatest number of LH-RH receptors was seen in the normal rats on day 35 of age and these were considerably increased by administration of 0.05 μg doses of D-Trp^6-LH-RH. In contrast, 1 μg D-Trp^6-LH-RH induced a clear lowering of

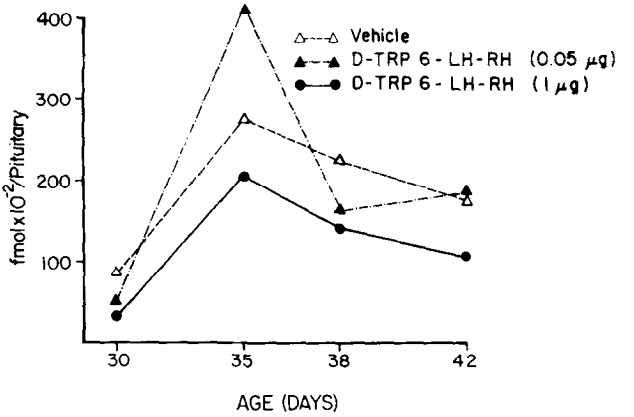
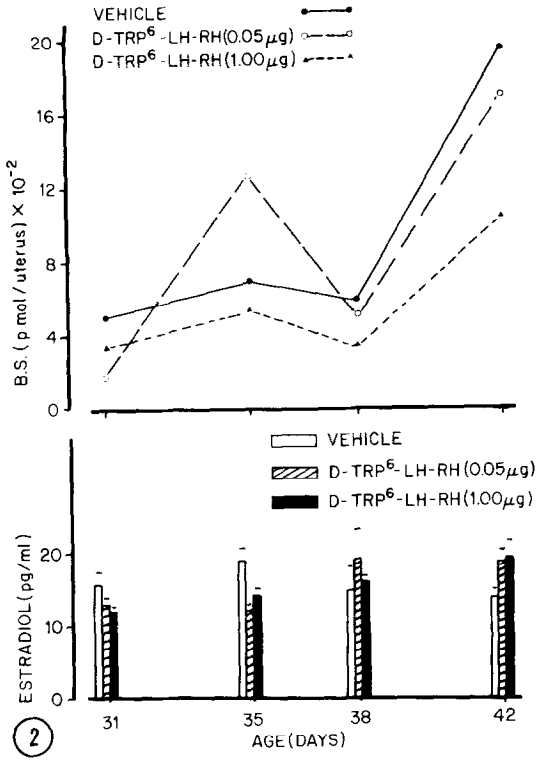


Fig. 1: Effect of D-Trp^6-LH-RH on LH-RH pituitary receptors. Pituitaries were obtained from intact immature female rats treated with the D-Trp^6-LH-RH or vehicle alone. The receptor was measured in the pituitary homogenate (5). The results represent the mean value ± SE of six points.

LH-RH pituitary receptors on all days studied, even 2 days after cessation of analog treatment (day 42).

Effect of D-Trp^6-LH-RH on the Serum Estradiol and its Uterine Binding Sites (Fig. 2). No significant changes on serum estradiol were seen in any of the studied groups. In the normal rats, no significant fluctuations in the uterine receptors for estradiol were observed between ages 31 to 38 days; however, a great increase at age 42 days was observed. A similar pattern on the uterine receptors for estradiol was seen when 0.05 μg D-Trp^6-LH-RH was given, the only difference being that a significant rise of those receptors on day 35 of age was found. On the contrary, 1 μg D-Trp^6-LH-RH induced a marked reduction in the uterine receptors for estradiol on all studied days, even on day 42 of age.

Direct Effect of D-Trp^6-LH-RH on Estradiol Uterine Receptors. The effect of D-Trp^6-LH-RH on estradiol uterine receptors in ovariectomized, hypophysectomized 35-day-old rats is shown in Fig. 3. A significant increase in the uterine weight and estradiol uterine receptors was induced by the estradiol treatment and the latter was enhanced when 0.05 μg D-Trp^6-LH-RH was given simultaneously. Surprisingly, both uterine weight and receptors were significantly reduced by 1 μg D-Trp^6-LH-RH.



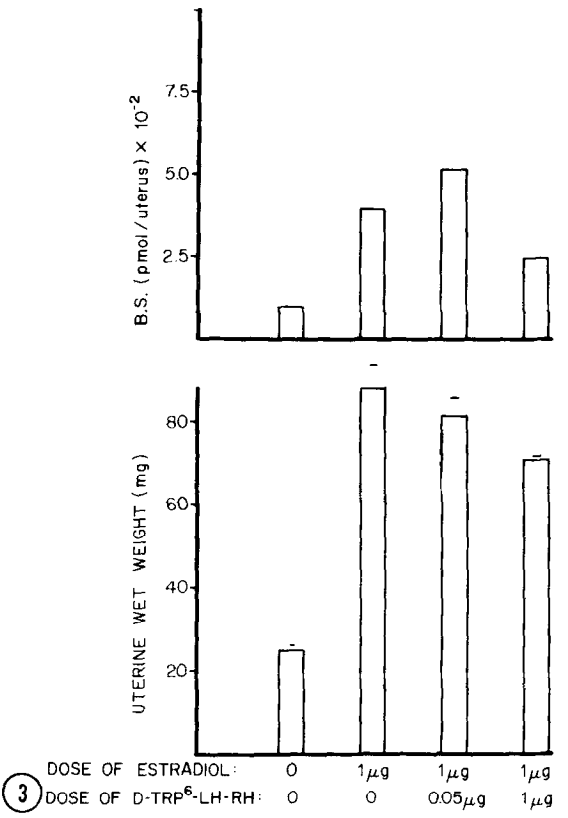


Fig. 2: Effect of D-Trp^6-LH-RH on the serum estradiol and its uterine binding sites. It was studied in immature female rats treated with the analog or vehicle alone at different days of age. Estradiol in serum was measured by radioimmunoassay (3) and the uterine binding sites were measured in the cytosol (6). Estradiol values represent the mean ± SE of six determinations per group and the binding sites triplicate determinations.

Fig. 3: Direct effect of D-Trp^6-LH-RH on estradiol uterine receptors. It was evaluated in the uterine cytosol of ovariectomized hypophysectomized rats on the 35th day of age treated with estradiol or with estradiol plus the analog. Uterine weight represents the mean of six determinations ± SE and uterine receptor triplicate determinations.

DISCUSSION

Previously, we have demonstrated that the superactive analogs of LH-RH possess prolonged LH- and FSH-releasing activities (10) and that the blockade of ovulation induced by the antagonists was parallel to their anti-LH/FSH releasing activities (7). In this paper, we show that the biological activity of both superactive and inhibitory LH-RH analogs correlated well with their receptor affinities. The ability of [D-Trp^6]-LH-RH

and [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH to displace LH-RH from its pituitary receptors, corroborated our previous work (5), however, in these experiments analog concentrations were reduced in order to better evaluate different potencies in the receptor assay. Comparable results for several LH-RH analogs have been reported by others (11).

Although it has been shown that the gonadotropin releasing activity of the superactive analogs correlated with their binding to the pituitary gland (12), effects on LH-RH pituitary receptor levels have not been reported previously. Here, we have observed that the alteration in numbers of LH-RH pituitary receptors depended on the amount of analog given to the animals-low doses increased and high doses reduced those receptors. These results can explain the significant depletion of pituitary gonadotropin content induced by the superactive analog (10).

Under our experimental conditions, no changes in serum estradiol were seen as was observed in pregnant rats (2). However, reductions in estradiol and progesterone in blood in adult female rats due to the chronic analog treatment have been described (13). This discrepancy with our results is probably due to the different experimental models and treatments.

The lowering of the ovarian and testicular LH-HCG receptor levels by LH-RH or the superactive analogs has been reported (13,14,15), but to our knowledge, no correlation between the superactive analog activity and uterine estradiol receptors has been studied. We observed an increase and a decrease in the number of those receptors with low and high doses of D-Trp^6-LH-RH, respectively. The increment seen in the uterine weight correlated with the uterotrophic effect produced in mice by the superactive analogs (16). It has been shown that other superactive analog exerts a pronounced inhibition of ovarian and uterine augmentation by HCG in immature rats (14), suggesting the possibility of an extrapituitary effect of these compounds, but it is difficult to prove that conclusion because intact animals were used in those experiments. We also observed that the high doses of D-Trp^6-LH-RH diminished

the uterine weight as well as the uterine receptors for estradiol in the intact animals. We demonstrated that this effect is exerted directly on the uterus, because similar results were obtained in the ovariectomized-hypophysectomized rats. This is the first evidence that LH-RH analogs have a direct effect on uterus. The possible mechanism of action of the D-Trp^{6}-LH-RH on uterus presumably involves the modification of the estradiol uterine receptors.

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