Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 687-693

REDUCTION OF TESTICULAR LUTEINIZING HORMONE/HUMAN CHORIONIC GONADOTROPIN RECEPTORS BY [D-Trp^6]-LUTEINIZING HORMONE RELEASING HORMONE IN HYPOPHYSECTOMIZED RATS

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Received June 27,1979

SUMMARY

Adult and immature male rats were hypophysectomized and injected daily with saline or 0.2 or 2 µg of superactive Luteinizing Hormone Releasing Hormone (LHRH) agonist, [D-Trp^6]-LHRH subcutaneously for seven days - with, or without, concomitant treatment of 1 IU Human Chorionic Gonadotropin(hCG) or 50 IU Pregnant Mare Serum. The administration of [D-Trp^6]-LHRH reduced Luteinizing Hormone/Human Chorionic Gonadotropin receptors in all cases. The magnitude of this reduction was dose-related. As small a dose as 0.2 µg of the peptide resulted in approximately a 72% reduction of the receptors. The results suggest a direct action of [D-Trp^6]-LHRH on the testis. It also indicated that reduction of testicular Luteinizing Hormone/Human Chorionic Gonadotropin receptors by the peptide is not necessarily due to the over-stimulation of Luteinizing Hormone (LH) release from the pituitary through a "down regulation" mechanism.

Although Luteinizing Hormone Releasing Hormone (LHRH) stimulates the release of both Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) from the pituitary in vivo and in vitro, the administration of large doses of LHRH or potent LHRH agonists - for a prolonged period results in gonadal involution in animals (1-5). Even in fairly small doses some of the superactive agonists induce paradoxical effects, such as prevention of implantation of fertilized ova (6); interruption of gestation (1-7); suppression of spermatogenesis (8); and reduction of ovarian (9) and testicular Luteinizing Hormone/Human Chorionic Gonadotropin (LH/hCG) receptors (10). These paradoxical effects were believed to be due to

Abbreviations: LH, Luteinizing Hormone; FSH, Follicle Stimulating Hormone; hCG, Human Chorionic Gonadotropin; LHRH, Luteinizing Hormone Releasing Hormone; PMS, Pregnant Mare Serum; sc, subcutaneously; NSB, non-specific binding.

0006-291X/79/190687-07$01.00/0

"down regulation", i.e., reduction of LH/hCG receptors resulting from overstimulation of LH release by an LHRH agonist (9,10,18).

In a previous study we compared [D-Trp^6]-LHRH and hCG effects on gestation in rats (6). Although both substances eventually blocked gestation, the appearance of the uterus of the [D-Trp^6]-LHRH - treated rat was distinctively different from that of the hCG-treated animal. [D-Trp^6]-LHRH treatment completely prevented the implantation of fertilized ova, as well as ballooning and fetal swelling of the uterus. On the other hand, some fetal swelling of the uterus - along with thickening of the wall - was observed in the rat treated with a large dose of hCG, but none of the fetuses survived full term. These findings suggest that the paradoxical effect of LHRH agonists cannot be fully accounted for by oversecretion of LH. Yoshinaga el.al. (7) reported that the paradoxical effect could not be reproduced by administration of large doses of LH.

The present study is to investigate if treatment with LHRH agonist reduces testicular LH/hCG receptors in the absence of the pituitary gland. It addresses the question: Is reduction of gonadal LH/hCG receptors by LHRH agonist induced by "down regulation", or is it caused by a direct effect of LHRH on gonadal receptors?

METHODS

Animals: Adult or immature male rats of CD strain from Charles River were housed in animal quarters which were equipped with controlled lighting (light: 0500 hrs. to 1900 hrs.) and controlled temperature (24 ± 20°C). Animals were fed on Purina Chow rat diets and water ad libitum. Experimental rats were hypophysectomized through the auditory canal under Nembutal (Abbott Lab., North Chicago,IL) anesthesia supplemented with ether.

Experiment 1: Young adult male rats weighing 180-200g were used. They were all hypophysectomized through the external auditory canal, and divided into the following four groups:

Group A: Received 0.2 ml 0.9% saline, subcutaneously(sc) daily for 7 days, starting on the day when the animal was hypophysectomized.

Group B: Similar to Group A, except that the animals received 1 IU hCG (human chorionic gonadotropin for injection, U.S.P. Ayerst Lab., New York, NY) sc every 2 days for 7 days. The first injection of hCG was given on the day of hypophysectomy.

Group C: Received daily sc injection of 2 µg [D-Trp^6]-LHRH/100g B.W. (Ayerst Lab., AY 25650) dissolved in 0.5ml 0.9% saline for 7 days, starting on the day of hypophysectomy.

Group D: Similar to Group C, except that in addition to [D-Trp^6]-LHRH, the animals also received 1 IU hCG in a manner similar to that of Group B.

Seven days after hypophysectomy, all animals were sacrificed by decapitation and testicular tissue was assayed for LH/hCG receptors.

Experiment 2: Immature male rats of 26 days of age were divided into two groups, A and B. The rats of Group A were injected with 50 IU Pregnant Mare Serum (PMS) (Ayerst Lab., Montreal, Canada) sc and hypophysectomized 67 hrs later. Group B did not receive any treatment before hypophysectomy. Each group was subdivided into three groups which were composed of A1,A2,A3,B1,B2,B3 and designated as follows:

A1 and B1: Injected with 0.2ml 0.9% saline sc daily at 0900 hr for 7 days, beginning on the day of hypophysectomy.

A2 and B2: Injected with 0.2ml 0.9% saline containing 0.2 µg [D-Trp^6]-LHRH sc.

A3 and B3: Injected with 2 µg [D-Trp^6]-LHRH sc daily for 7 days, beginning on the day of hypophysectomy.

All animals were sacrificed 7 days after hypophysectomy, i.e., one day after the last injection of saline or [D-Trp^6]-LHRH.

LH/hCG receptor assay: The testicular LH/hCG receptor assay reported by Catt et.al. (11) was modified for use in the present studies. After decapitation of each animal, one testis was dissected, decapsulated and weighed. It was then homogenized in 2ml ice-cold 0.25 M sucrose, 5 mM $MgCl_2$ 0.1 M Tris-HCl buffer, pH 7.4 using 15 strokes in a Teflon-glass Potter homogenizer.

The homogenate was decanted into 25ml of the sucrose-containing Tris-HCl buffer and centrifuged at 13,000 rpm for 15 minutes. The pellet thus obtained was resuspended in 0.1% bovine serum albumin, 5mM $MgCl_2$, Tris-HCl buffer, pH 7.4 to obtain a concentration of 10mg tissue per 100 µl. The assay was performed using 10mg equivalents of tissue per tube and 1-2 ng ^{125}I-hCG in a total volume of 0.5 ml. Non-specific binding was assessed by addition of 2 µg bovine LH (NIH-B-10), which a preliminary determination indicated was sufficient to saturate receptor sites. By this means, non-specific binding (NSB) ranged from 0.1 to 0.5% of total bound radioactivity. Incubation was carried out in duplicate at room temperature for 16-20 hours. Reaction was stopped by addition of 1 ml ice-cold bovine serum albumin(BSA) - containing Tris-HCl buffer.

The incubate was then centrifuged at 3000 rpm at 4°C for 30 min. The precipitate was washed and recentrifuged, then counted for radioactivity by a Searle Autoganna Counter, Model 1285 with an efficiency of 70%. The specific binding was calculated by subtracting non-specific from the total bound radioactivity for each homogenate, expressed in cpm per mg of tissue.

^{125}I-hCG was prepared by radioiodinating 5 µg hCG (Serono Labs, Boston, Mass. 15,000 IU/mg) with 1 mCi Na^{125}I, using a lactoperoxidase method as described by Miyachi et.al. (12). The reaction mixture was purified on a Sephadex G-100 column, 1 X 50 cm, using 0.1 M Tris-HCl buffer, pH 7.4 as eluent. The specific activity of ^{125}I-hCG ranged from 40-80 µCi/µg as calculated from the elution pattern.

RESULTS

As shown on Table 1, daily injection of 2 µg [D-Trp^6]-LHRH for 7 days in hypophysectomized adult rats significantly ($p < 0.01$) reduced LH/hCG binding sites of the testis, but did not affect the testicular wieght. Administration of 1 IU

TABLE 1: Effect of treatment with hCG and [D-Trp^6]-LHRH on testicular LH/hCG receptor in hypophysectomized adult rats.

Group	Treatment	Mean Body Wt. + SE (g)	Mean Testicular Wt.[4] + SE (mg)	Testicular LH/hCG receptor[5] (cpm / 10mg)
A	Saline[1]	170 ± 11.84 (4)[3]	628 ± 10.7 (4)	1264 ± 275.2 (4)
B	Saline + hCG[2]	155 ± 6.5 (4)	901 ± 40.5 (4)	801 ± 127.0 (4)
C	[D-Trp^6]-LHRH	163 ± 12.6 (4)	656 ± 40.8 (4)	443 ± 103.5 (4)
D	[D-Trp^6]-LHRH + hCG	161 ± 3.7 (4)	872 ± 38.2 (4)	213 ± 100.2 (4)

1 Saline or 2 µg [D-Trp^6]- LHRH / 100 g B.W. was injected sc daily for seven days, starting on the day of hypophysectomy.

2 1 IU hCG was injected sc every 2 days for seven days, starting on the day of hypophysectomy.

All animals were sacrificed on the day following that of the last injection.

3 Number in parenthesis indicates number of rats.

4 Testicular weight:
A vs. B $p < 0.01$
C vs. D $p < 0.01$
A vs. C Not significant
B vs. D Not significant

5 LH/hCG receptors:
A vs. B Not significant
A vs. C $p < 0.01$
A vs. D $p < 0.01$
B vs. D $p < 0.05$

hCG every 2 days for seven days increased the testicular weight ($p < 0.01$), and decreased mean LH/hCG binding sites; but the latter difference was not significant. Daily administration of [D-Trp^6]-LHRH concomitant with hCG further reduced LH/hCG binding sites. The number of LH/hCG binding sites in this last group was smaller ($p < 0.05$) than that in the saline-hCG group. The treatment with the peptide alone reduced the number of LH/hCG binding sites to 1/3 to 1/4 of the respective control value (Table 1).

Similarly, treatment with [D-Trp^6]-LHRH decreased testicular LH/hCG binding sites in hypophysectomized immature rats - whether pretreated with PMS, or not (Table 2). The larger dose of the LHRH agonist induced a greater reduction of

TABLE 2: Effect of PMS and different doses of [D-Trp^6]-LHRH on testicular LH/hCG receptor in hypophysectomized immature rats.

Group	Treatment	Mean Body Wt. ± SE (g)	Mean Testicular Wt. ± SE (mg)	Testicular LH/hCG receptor[4] (cpm / 10mg)
A1	PMS[2] + Saline[1]	90 ± 2.8 (6)[3]	360 ± 25.0 (6)	34677 ± 1385.8 (3)
A2	PMS + 0.2 µg [D-Trp^6]-LHRH	89 ± 1.5 (8)	286 ± 20.4 (8)	9571 ± 1066.4 (8)
A3	PMS + 2 µg [D-Trp^6]-LHRH	86 ± 2.2 (7)	325 ± 18.4 (6)	4999 ± 448.6 (5)
B1	Saline	84 ± 3.2 (5)	169 ± 14.8 (5)	22792 ± 1330.8 (5)
B2	0.2 µg [D-Trp^6]-LHRH	79 ± 4.0 (5)	186 ± 41.9 (5)	10070 ± 1317.5 (5)
B3	2 µg [D-Trp^6]-LHRH	80 ± 2.5 (6)	193 ± 10.1 (5)	6567 ± 666.2 (5)

1 Saline or [D-Trp^6]-LHRH was injected sc daily for seven days, starting on the day when the animal was hypophysectomized.

2 50 IU PMS was injected 67 hours before hypophysectomy.

3 Number in parenthesis indicates number of rats per group.

4 LH/hCG receptors:
A1 vs. A2 : $p < 0.01$
A1 vs. A3 : $p < 0.01$
A2 vs. A3 : $p < 0.01$

B1 vs. B2 : $p < 0.01$
B1 vs. B3 : $p < 0.01$

binding sites, but the difference did not reach a significant level in the B groups. Injection 50 IU PMS 67 hours before hypophysectomy caused an increase in the testicular weight as well as an increase in LH/hCG binding sites (A1 vs. B1 in Table 2). However, PMS did not affect the LH/hCG receptor-reducing effect of [D-Trp^6]-LHRH.

DISCUSSION

The present results clearly indicate that the administration of [D-Trp^6]-LHRH results in a reduction of testicular LH/hCG receptors in the absence of the pituitary gland. This means that the decrease in LH/hCG receptors induced by LHRH agonist is not necessarily caused by over-stimulation of LH release

from the pituitary gland through a "down regulation" mechanism. (9,19,18).

This reduction of LH/hCG receptors by the LHRH agonist was also dose-related. As small a dose as 0.2 μg [D-Trp^6]-LHRH induced a dramatic decrease of the receptors, especially in hypophysectomized immature rats pretreated with PMS, a 72% reduction being observed. It is likely that doses less than 0.2 μg would be sufficient to significantly reduce testicular LH/hCG receptors in the absence of the pituitary. The attempt to treat tertiary hypogonadism by LHRH agonist in fairly small doses could directly reduce gonadal LH/hCG receptors, thereby nullifying the effect of enhanced LH release.

Although the data strongly suggests that reduction of LH/hCG receptors resulted from a direct effect on the testis, confirmatory evidence is still lacking. The presence of receptors for LHRH or its agonist in the ovarian tissues was reported (13). Our recent autoradiographic study revealed that exogenously administered ^{125}I-labeled [D-Trp^6]-LHRH was taken by the testicular tissue. The uptake was competitively suppressed by prior administration of a large dose of unlabeled agonist. Since such competitive inhibition was observed in the pituitary, but not in the other organs (13), the binding of the tracer by the testis is likely to be specific. It is possible that [D-Trp^6]-LHRH binds with the testicular receptor, initiating intracellular processes which lead to a reduction of LH/hCG receptors. However, the physiological significance of the testicular and ovarian receptors for LHRH and its agonists remains unknown.

The presence of adrenal LHRH receptors has also been reported (23). It is also possible that the agonist directly affects the adrenal, altering adrenal steroidogenesis, and thereby influencing testicular LH/hCG receptors.

Acknowledgements. This work was supported in part by USPHS research grants AM 09094, AM 07467, and Veterans Administration.

Addendum. During the preparation of this manuscript, Hsueh and Erickson reported that LHRH and its agonist inhibited FSH-induced increase of estrogen and progesterone porduction *in vitro* by rat ovarian granulosa cells. They also stated that the agonists inhibited FSH-induced changes in ovarian function in hypophysectomized rats *in vivo*. (Hsueh, A.J.W. and Erickson, G.F., Science 204: 854-855, 1979). These findings are compatible with the results of this present study.

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