INHIBITION OF TESTICULAR LUTEINIZING HORMONE RECEPTOR LEVEL BY TREATMENT WITH A POTENT LUTEINIZING HORMONE-RELEASING HORMONE AGONIST OR HUMAN CHORIONIC GONADOTROPIN

Claude Auclair, Paul A. Kelly, Fernand Labrie, David H. Coy*, and Andrew V. Schally*

Medical Research Council Group in Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada, and *Department of Medicine, Tulane University School of Medicine, and VA Hospital, New Orleans, La 70112

Received April 21,1977

SUMMARY: Treatment of adult male rats with a potent luteinizing hormone-releasing hormone agonist, [D-Leu^6, Des-Gly-NH_2^{10}] LHRH ethylamide or human chorionic gonadotropin for one week caused a marked decline in luteinizing hormone receptors in testicular tissue. Testicular weight was also significantly decreased by these treatments while plasma testosterone levels were decreased by treatment with the LHRH agonist and increased after hCG. A single injection of the LHRH agonist or hCG also induced a long-lasting loss of testis LH binding sites with receptor levels returning to control values at 8 days. These findings indicate than an elevation of endogenous LH, whether induced by single or repeated injection of a potent LHRH agonist, is capable of down regulating testicular LH receptors with a resultant long-term effect of reduced testicular size and decreased plasma testosterone concentration.

A marked loss of LH receptors (1, 2) and steroidogenic response to gonadotropins (2) has been observed in the rat after systemic administration of ovine LH or hCG. This ability of gonadotropins to induce loss of their own receptors is analogous to the effect of insulin, thyrotropin-releasing hormone, growth hormone and catecholamines on the level of their own receptor in respective target tissues (3-8). This concept becomes extremely important in view of the fact that LHRH and its analogues are currently used in the treatment of oligospermia and male infertility. In the present report, we have studied the effect of single and repeated injections of [D-Leu^6, Des-Gly-NH_2^{10}] LHRH ethylamide or hCG on the level of LH/hCG receptors in rat testis.

Abbreviations used: LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; hCG, human chorionic gonadotropin.

ISSN 0006-291X

MATERIALS AND METHODS

Animals: Adult male Sprague-Dawley rats weighing 185-205 g upon arrival were obtained from Canadian Breeding Farms, St. Constant, Quebec. Animals were housed 2 per cage in a temperature (20-22°C)- and light (14-h light, -10-h darkness)-controlled room and given food and water ad libitum.

Hormones: Purified hCG (CR 119, 11,6000 IU/mg) was generously supplied by the Center for Population Research of the NICHHD, NIH. For in vivo treatment, a preparation of hCG (APL, approximately 2600 IU/mg) obtained from Ayerst was used. Ovine LH (NIH-LH-S19 1.01 x NIH-LH-S1) was a gift of the National Pituitary Agency, NIH. [D-Leu^6, Des-Gly-NH_2^{10}] LHRH ethylamide was synthesized in a solid-phase system and purified as described (9).

Treatment with the LHRH agonist or hCG: In the first experiment, the animals (8 per group) were injected with either 0.6, 3, 15 or 75 µg of the LHRH agonist, hCG (50 IU) or the vehicle alone (0.15M NaCl) three times a day (at 0800, 1600 and 2400 h) for 7 days. Animals were sacrificed 16-18 h after the last injection of the LHRH analogue or hCG. In a second experiment, the effect of single injection of the LHRH analogue (125 µg) or hCG (200 IU) was studied at the indicated time intervals after injection. In the two studies, animals were killed by decapitation between 0800 and 1000 h, testis removed and plasma collected for testosterone RIA.

LH/hCG receptor assay: Decapsulated testes were homogenized in 40 ml of 0.1M Tris-HCl, pH 7.4, 0.25 M sucrose, 5 mM $MgCl_2$ with a Sorval tissue homogenizer at a speed setting of 10 for 15 sec. After centrifugation of the homogenate at 20,000 xg for 15 min, the pellet was resuspended in 40 ml of buffer and recentrifuged. The pellet was then suspended in 3 ml of buffer (without sucrose but with 0.1% bovine serum albumin) and appropriate dilutions made so that each assay tube received 5 mg equivalents of tissue. For iodination, 500 µCi [^{125}I], 2.5 µg of hCG and 1000 ng of chloramine T were incubated on ice for 2-3 min. The hormones were then repurified on a column (0.9 x 100 cm) of Sephadex G-100. As calculated by isotope recovery, the specific activity was 41.6 µCi/µg.

LH/hCG binding was measured by duplicate incubation for 14-16 h at room temperature of 100 µl of the testis suspension with 200 µl buffer, 100 µl of [^{125}I] hCG (approximately 100,000 cpm or 40 fmol) in the presence or absence of an excess of unlabeled hormone (20 µg/ml ovine LH) in 12 x 75 mm glass tubes. The reaction was stopped by dilution with 4 ml ice-cold buffer and the mixture centrifuged at 2000 rpm for 15 min. The supernatant was decanted and the pellets counted in a LKB autogamma spectrometer with a counting efficiency of 74%. Difference of the cpm bound in the absence and presence of excess unlabeled hormone (specific binding) was expressed as fmoles of bound hormone.

Testosterone assay: Plasma concentrations of testosterone were measured by a specific double-antibody RIA. Plasma was extracted with benzene and the extract assayed directly using an antibody developed against testosterone-7α-undecanoate and [^{125}I]testosterone-7α-butyric tyrosine methyl ester. The limits of the assay were 5 and 5000 pg testosterone per tube. Calculations were performed as described (10).

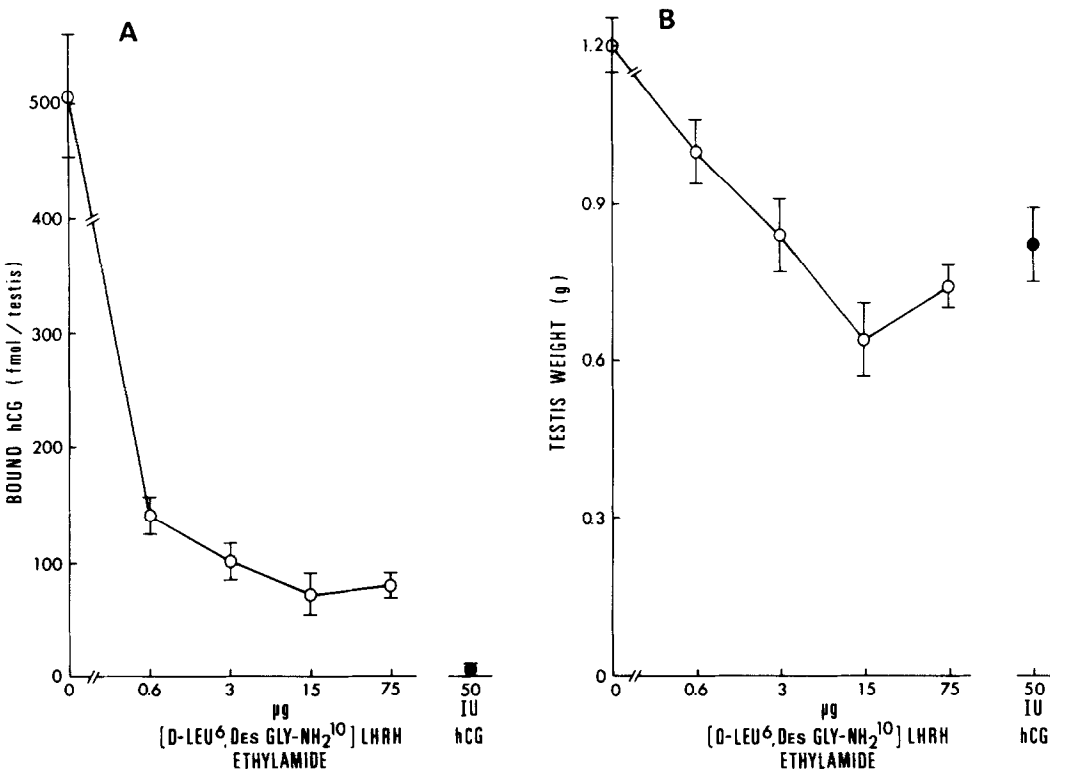

Fig. 1. Effect of treatment with increasing doses of [D-Leu^6, Des-Gly-NH$_2$ ^{10}] LHRH ethylamide (o) or hCG (•) on binding of [^{125}I] hCG to testicular LH/hCG receptors (A) and testis weight (B) in adult male rats. Animals were injected three times a day with the indicated doses of the LHRH agonist or hCG for one week.

RESULTS

Long-term administration of [D-Leu^6, Des-Gly-NH$_2$ ^{10}] LHRH ethylamide caused a marked reduction of LH/hCG binding sites in testicular tissue from 507 ± 53 to 140 ± 16 fmol/testis (Fig. 1A). A greater decrease was even seen at higher doses of the LHRH analog. Injection of hCG caused an almost complete loss of LH/hCG binding sites. Testis weight was also significantly reduced by these treatments (Fig. 1B).

From the data in Fig. 1, it can be calculated that a decline in LH/hCG receptor levels was also observed when hCG binding was expressed on a basis of testis weight. Also a 55 to 75% reduction of plasma testosterone levels was observed after treatment with the LHRH analogue while increased plasma androgen levels were found after hCG administration (Table I).

TABLE I

Testicular LH/hCG receptor levels and plasma testosterone concentration in male rats injected with increasing concentrations of [D-Leu^6, Des-Gly-NH$_2$^{10}] LHRH-ethylamide or hCG[a, b].

Treatment	Dose	Bound hCG fmol/g testis	Plasma testosterone ng/ml
Control	0	438 ± 55	6.5 ± 1.0
[D-Leu^6, Des-Gly-NH$_2$^{10}] LHRH	0.6 µg	137 ± 13**	2.9 ± 0.4
	3 µg	118 ± 18**	2.5 ± 0.3*
	15 µg	132 ± 34**	2.2 ± 0.3*
	75 µg	108 ± 11**	1.6 ± 0.2*
hCG	50 IU	5 ± 2**	26.6 ± 3.0**

*, $p < 0.05$;

**, $p < 0.01$ (exp vs control)

[a] Animals were injected subcutaneously 3 times a day for 7 days with the indicated doses of the LHRH agonist or 50 IU of hCG

[b] Calculations based on a molecular weight for hCG of 37,900 (15, 16)

A single injection of 125 µg [D-Leu^6, Des-Gly-NH$_2$^{10}] LHRH-ethylamide or 200 IU hCG induced a rapid decline in testis LH/hCG receptor levels (Fig. 2A). The effect was apparent at 12h, remained maximal until 3 days and progressively increased toward normal values thereafter. After a slight increase at 12 h, testicular weight declined steadily in both groups and remained low up until 8 days (Fig. 1B).

It can be seen in Table II that the changes of LH/hCG receptor levels followed the same pattern when expressed per g testis. After a small increase 12 h after injection of [D-Leu^6, Des-Gly-NH$_2$^{10}]

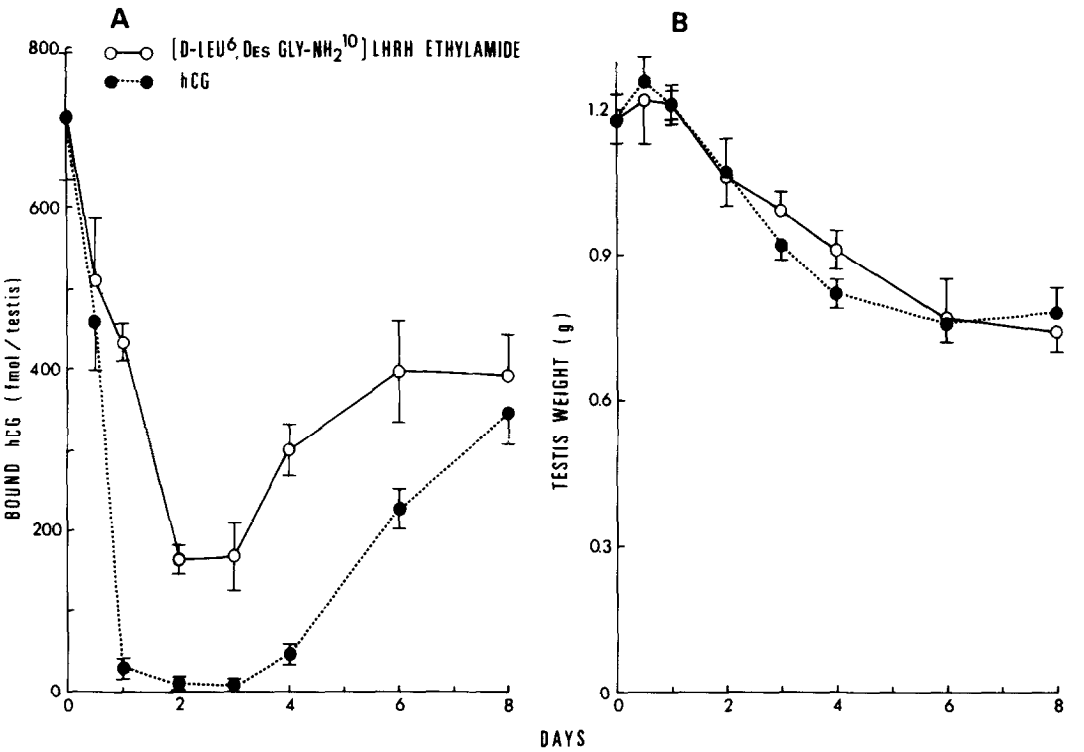

Fig. 2. Time-course of the effect of a single injection of [D-Leu^6-Des-Gly-NH_2 ^{10}] LHRH ethylamide (125 μg) or hCG (200 IU) on binding of [^{125}I] hCG to testicular LH/hCG receptors (A) and testis weight (B) in adult male rats.

LHRH ethylamide, plasma testosterone levels were decreased at 3 days and returned to normal at later time intervals (Table 2). A single injection of hCG led to a sustained elevation of plasma testosterone levels up to 3 days after its administration with a small decrease at 5 and 6 days and return to normal levels at 8 days.

DISCUSSION

The present data clearly demonstrate that single or repeated injection of the potent LHRH agonist [D-Leu^6, Des-Gly-NH_2 ^{10}] LHRH ethylamide can lead to a marked and sustained loss of testicular LH receptors. A single injection of 125 μg of the peptide analog depleted testicular LH receptors down to 25% of control levels while an effect of similar magnitude was measured after injection of 0.6 μg of the peptide 3 times a day for one week. The time-course of the effect of the LHRH agonist is very similar to that

TABLE II

Time-course of the response to a single injection of [D-Leu^6, Des-Gly-NH_2^{10}] LHRH ethylamide or hCG on LH testicular receptor levels and plasma testosterone concentration in adult male rats

Treatment	Days after injection	Bound hCG fmol/g testis	Plasma testosterone ng/ml
Control	0	596 ± 50	8.2 ± 1.7
[D-Leu^6, Des-	0.5	409 ± 42**	13.3 ± 2.5*
Gly-NH_2^{10}]LHRH	1	361 ± 18**	8.2 ± 0.9
ethylamide	2	156 ± 16**	5.5 ± 0.4
(125 µg)	3	168 ± 32**	8.2 ± 1.9
	4	332 ± 42**	8.5 ± 2.0
	6	488 ± 66*	6.9 ± 1.1
	8	525 ± 55	7.0 ± 1.4
hCG	0.5	364 ± 47**	26.4 ± 2.5**
(200 IU)	1	24 ± 8**	17.0 ± 2.1**
	2	11 ± 5**	13.9 ± 1.6*
	3	11 ± 5**	13.5 ± 2.4*
	4	55 ± 13**	6.1 ± 0.8
	6	298 ± 29**	6.2 ± 0.6
	8	443 ± 37**	8.4 ± 1.0

*, $p < 0.05$;

**, $p < 0.01$ (exp vs control)

obtained in these (Figs 1 and 2) and previous experiments using hCG or ovine LH (1, 2). Although the effects measured after treatment with hCG could partly reflect occupancy of binding sites by the exogenous hormone, the delayed and sustained effects measured

after a single injection of the LHRH analog clearly indicate a negative regulation of LH receptors by endogenous LH. As observed previously with ovine LH and hCG (1, 2), the present data obtained with the LHRH analog reveal that maximal receptor loss was delayed 24 to 48 h after maximal plasma LH levels.

Since plasma LH levels are likely to be back to normal within 12 h after injection of [D-Leu^6, Des-Gly-NH$_2^{10}$] LHRH ethylamide (11), the findings (Fig. 2, Table II), of low levels of testicular LH receptors up to 4 days after injection of the LHRH analogue indicate slow recovery of LH receptors. In agreement with those data, a sustained loss of testicular LH receptors has been observed after injection of ovine LH (1) or a small dose of hCG, which led to about only 8% occupancy of the testis LH receptors (2). Such data suggest that changes of plasma LH levels induced by endogenous or exogenous LH-releasing peptide(s) or exogenous gonadotropins can lead to sustained and marked alterations of the population of testis LH receptors.

Since spare LH receptors in excess of the number required for maximal testosterone secretion are present in the testis (12, 13), a loss of LH receptors would not be expected to be automatically followed by a parallel decrease of androgen secretion. It is interesting to note that a 75% decrease of testicular LH receptors after a 7-day treatment with the LHRH analog was accompanied by a 50 to 75% decrease of plasma testosterone levels and a reduction of testis and prostate weight. The role of possible changes of endogenous LH secretion has however not been assessed. These findings could provide an explanation for the absence of elevated plasma testosterone levels in men having hCG-producing tumors (14). The finding of normal plasma testosterone levels 3 days after single injection of the LHRH agonist in the presence of severely depressed LH receptors suggests the ability of the hypophyso-gonadal system to compensate for partial LH receptor loss. A dissociation between loss of LH receptors and the testosterone response to hCG in vitro has also been observed after the single injection of 10 IU of hCG in the rat while a higher dose (200 IU) led to an almost complete inhibition of both LH receptors levels and steroidogenic response to the gonadotropin (2).

Although the mechanisms of testicular receptor loss and the influence of treatment with the LHRH analogue on endogenous LHRH

secretion, LH responsiveness to LHRH, steroidogenesis and other relevant hormonal secretions remain to be elucidated, the present data indicate that the successful long-term therapy with LH-releasing peptides will greatly benefit from careful evaluation of optimal dose and time of administration of the drug.

REFERENCES

1. Hsueh, A.J.W., Dufau, M.L. and Catt, K.J. (1976) Biochem. Biophys. Res. Commun. 72, 1145-1152.
2. Hsueh, A.J.W., Dufau, M.L., and Catt, K.J. (1977) Proc.Natl Acad. Sci. 74, 592-595.
3. Kahn, C.R., Neville, D.M., and Roth, J. (1973) J. Biol. Chem. 248, 244-250.
4. Gavin, J.R., Roth, J., Neville, D.M., De Meyts, P. and Buell, D.N. (1974) Proc. Natl Acad. Sci. 71, 84-88.
5. Hinkle, P.M. and Tashjian, H.H. (1976) Biochemistry 14, 3845-3851.
6. Lesniak, M.A., and Roth, J. (1976) J. Biol. Chem. 251, 3730-3739.
7. Lesniak, M.A., Roth, J., Gorden, P., and Gabin, J.R. (1973) Nature New Biol. 241, 20-21.
8. Mukherjee, C., Caron, M.G., and Lefkowitz, R.J. (1975) Proc. Natl Acad. Sci. 72, 1945-1949.
9. Coy, D.H., Coy, E.J., Schally, A.V., Vilchez-Martinez, J.A., Debeljuk, L., Carter, W.H., and Arimura, A. (1974) Biochemistry 13, 323-326.
10. Drouin, J., and Labrie, F. (1976) Endocrinology 98, 1444-1451.
11. Vilchez-Martinez, J.A., Coy,D.H., Arimura, A., Coy, E.J., Hirotsu, Y., and Schally, A.V. (1974) Biochem. Biophys. Res. Commun. 59, 1226-1232.
12. Mendelson, C., Dufau, M.L., and Catt, K.J. (1975) J. Biol. Chem. 250, 8818-8823.
13. Catt, K.J., and Dufau, M.L. (1973) Adv. Exptl. Med. Biol. 36, 379-418.
14. Kirschner, M.A., Wider, J.A., and Ross, G.T. (1970) J. Clin. Endocrinol. Metab. 30, 504-511.
15. Bellisario, R., Carlsen, R.B., and Bahl, O.P. (1973) J. Biol. Chem. 248, 6796-6809.
16. Carlsen, R.B., Bahl, O.P. and Swminathan, N. (1973) J. Biol. Chem. 248, 6810-6827.