Plasma Luteinizing Hormone and Follicle Stimulating Hormone Responses to Luteinizing Hormone-Releasing Hormone in Patients with Hypogonadism

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Synopsis

Plasma LH and FSH responses to the intravenous injection of synthetic LH-RH were studied in 18 normal males and in 24 patients with hypogonadism of various etiologies. Intravenous injection of 100, 200 and 400 μ g of LH-RH into normal adult males caused a dose-dependent rise in plasma LH and FSH. Plasma LH rose significantly in all subjects after 100 μ g of LH-RH, whereas 200 ~ 400 μ g were necessary to achieve a consistent rise in plasma FSH.

In 7 patients with hypergonadotropic hypogonadism, plasma LH and FSH levels were elevated with a marked and sustained response to 100 μ g of LH-RH.

In 9 patients with isolated gonadotropin deficiency, plasma LH and FSH levels were either undetectable or low and their responses to 100 μ g of LH-RH were absent or limited.

In 5 of these patients, intravenous infusion of 400 μ g of LH-RH for 7 to 21 successive days improved plasma LH and FSH responsiveness to LH-RH, whereas the infusion for 2 days in 2 patients was not effective.

In 8 patients with failure of sexual maturation due to organic hypothalamo-hypophyseal lesions, plasma LH and FSH responses to 100 μ g of LH-RH were normal in one and low or absent in the remaining patients. In one patient with ectopic pinealoma, a diminished response of LH and FSH to LH-RH was normalized after 7 successive days treatment with the intravenous infusion of 400 μ g of LH-RH.

Synthetic LH-RH test is a useful tool for the differential diagnosis of hypogonadism. It is not always possible, however, to differentiate the hypothalamic from pituitary lesions by a single LH-RH test, because diminished response to LH-RH may indicate either pituitary lesions or pituitary hyporesponsiveness due to long-standing deficiency of endogenous LH-RH. In this regard, prolonged LH-RH stimulation test may help to differentiate between hypothalamic and pituitary lesions.

Hypogonadism is known to be caused by either primary defects in the gonads or deficient secretion of gonadotropins from the pituitary gland. Since gonadotropin secretion is regulated by the hypothalamus via the release of neurohumoral substances into the pituitary portal vessels, gonadotropin deficiencies can be classified further into 3 categories: the pituitary lesion, the hypothalamic lesion and both.

The hypothalamic releasing hormone for luteinizing hormone (LH) and possibly for follicle-stimulating hormone (FSH) has recently been isolated and shown to be a decapeptide.

This luteinizing hormone-releasing hormone (LH-RH) has now been synthesized and made available for the study of pituitary gonadotropin reserve. The aim of the present study was to establish whether the synthetic LH-RH test is useful for the differential diagnosis of hypogonadism of various etiologies.

Material and Methods

Subjects

Normal subjects used as a control were 13 male volunteers, aged 18 to 32, with apparently normal sexual function. Seven patients with hypergonadotropic hypogonadism studied in the present experiment consisted of 2 males with Klinefelter's syndrome, 2 males with germinal cell aplasia, 2 females with Turner's syndrome and a female with possible Turner's syndrome. Nine patients with isolated gonadotropin deficiency were also studied. They had normal pituitary hormone secretory capacity except for gonadotropin, as evaluated by the insulin-induced hypoglycemia and synthetic thyrotropin-releasing hormone (TRH) tests. Two of them, a male and a female, were siblings born from consanguineous marriage. One male patient had typical signs of Laurence-Moon-Biedle's syndrome. A female patient, aged 44, had poorly developed secondary sex characteristics with history of irregular menses from age 26 to 38. The remaining patients, 4 males and a female, were sporadic cases of hypogonadotropic hypogonadism with the typical eunuchoid stature. Eight patients with failure of sexual maturation due to organic hypothalamo-hypophyseal lesions were also studied. Five of them had tumors in the hypothalamo-hypophyseal region and 2 had cured tuberculous meningitis. The remaining patient had diabetes insipidus, short stature and poorly developed secondary sex characteristics, although the etiology was not determined yet.

Test procedures

As the standard test procedure, 100 μ g of the synthetic LH-RH dissolved in 10 ml of saline was given to the recumbent fasting subjects at 9:00 a.m. by rapid intravenous injection. In normal males, 200 μ g and 400 μ g of LH-RH were given also in a similar way. To test pituitary gonadotropin reserve further, 400 μ g of LH-RH dissolved in 500 ml of saline was infused intravenously over a period of 2 hours. The infusion of LH-RH was repeated in some patients on 2 to 21 successive days.

Blood was drawn from the antecubital vein by repeated venipuncture before and 15, 30, 45, 60, 90 and 120 minutes after the injection of LH-RH. Plasma was separated by centrifugation and stored at -20°C .

Hormone assays

Plasma LH and FSH levels were determined by double-antibody radioimmunoassay. Purified human pituitary LH (LER 960) and human pituitary FSH (LER 1366) as well as anti-LH and anti-FSH sera were

generously supplied by the National Pituitary Agency and NIAMD. Iodination of the hormones was performed by the method of Greenwood *et al.* (Greenwood *et al.*, 1963). 2nd-IRP-HMG were used as a standard preparation. In some experiments, purified LH and FSH as well as anti-LH and anti-FSH sera provided by Calbiochem, Limited, were used. Results obtained by both radioimmunoassay systems were almost identical. Details of the procedure are published elsewhere (Imura *et al.*, 1973). Minimal detectable values were estimated to be approximately 2 mIU/ml in all LH and FSH radioimmunoassays.

Results

Plasma LH and FSH responses to LH-RH in normal subjects

Plasma LH and FSH responses to the intravenous injection of 100 μ g, 200 μ g and 400 μ g of LH-RH were studied in 9, 4 and 5 normal males, respectively. Some subjects received 2 different doses of LH-RH with an interval of at least one week, whereas others were used only for one experiment.

Figure 1 illustrates the mean LH and FSH levels following three doses of LH-RH. Basal plasma LH and FSH levels in normal subjects were 4.5 to 15.5 and less than 2.0 to 7.5 mIU/ml, respectively. Following the injection of 100 μ g of LH-RH, plasma LH rose significantly, reaching peak levels ranging from 35.0 to 104.0 mIU/ml within 30 min. Plasma FSH rose slightly and gradually in most of the subjects, with peak values of 4.9 to 18.2 mIU/ml.

Intravenous injection of 200 and 400 μ g of LH-RH produced more marked rise in plasma LH and FSH than did 100 μ g of the peptide.

Plasma LH and FSH responses to LH-RH in patients with primary gonadal failure

Plasma LH and FSH levels were measured following a single injection of 100 μ g of LH-RH in 7 patients with hypergonadotropic hypogonadism. Basal plasma LH and FSH levels ranged from 17.5 to 66.0 mIU/ml and from 38.6 to 124.8 mIU/ml, respectively.

Intravenous injection of 100 μ g of LH-RH

Fig. 1. Plasma LH and FSH responses to 100, 200, 400 μ g of LH-RH in normal males. Number in parenthesis represents number of the normal subjects tested. Mean $\pm$ SEM are shown.

elicited a marked rise in plasma LH with peak values ranging from 95.0 to 290 mIU/ml, as shown in Figure 2. Although the ratio of peak to basal levels, 2.4 to 12.0, was comparable to that in normal subjects, elevated plasma LH levels were much more sustained. Plasma FSH rose slightly and gradually, and peak levels were 1.3 to 3.9 times the basal levels. This ratio is also identical to that of normal subjects.

Plasma LH and FSH responses to LH-RH in patients with isolated gonadotropin deficiency (Table 1)

Basal plasma LH levels were not detectable in 6 of 9 patients with isolated gonadotropin deficiency, and plasma FSH levels were undetectable in 7 of these 9. Table 1 shows plasma LH and FSH levels after the injection of 100 μ g of LH-RH. No significant change in plasma LH was observed in a female patient with familial gonadotropin deficiency (Case 5). The remaining 8 patients showed a rise in plasma LH in response to LH-RH, although the plasma LH rise was lower than that in normal subjects. Plasma FSH levels were virtually unchanged in 4 patients and slightly increased in the remainder.

Fig. 2. Plasma LH and FSH responses to 100 μ g of LH-RH in 7 patients with hypergonadotropic hypogonadism. Shaded areas represent normal peak ranges. Note that logarithmic values of plasma LH and FSH are plotted on the ordinate.

Table 1. Plasma LH and FSH responses to 100 μ g of LH-RH in 9 patients with isolated gonadotropin deficiency.

Case	Name	Age	Sex	0	15	30	45	60	90	120	0	15	30	45	60	90	120
				LH	mIU/ml							FSH	mIU/ml				
					min.								min.				
1	H.Y.	23	M.	<*	10.2	15.0	10.0	6.0	<	4.2	<	<	<	2.5	2.5	<	<
2	M.Y.	21	M.	<	2.5	6.5	4.0	13.0	6.5	5.5	<	<	<	<	<	<	<
3	T.H.	33	M.	2.8	6.7	8.4	13.9	13.3	18.2	15.8	<	<	<	4.0	6.3	6.5	2.7
4	K.F.	19	M.	<	6.5	3.0	4.0	6.0	6.0	7.5	<	<	<	<	<	<	<
5	S.H.	28	F.	<	<	<	<	<	2.3	2.6	<	<	<	<	<	<	<
6	Y.Y.	22	M.	<		8.9		8.9		19.6	<		2.6		3.4		5.8
7	S.Y.	44	F.	4.1	4.9	6.2	5.2	4.7	4.2	4.0	6.1	5.3	7.1	7.3	9.2	9.6	11.5
8	M.I.	19	F.	4.2		15.8	15.0	11.0	3.8	3.8	8.8	7.4	7.2	9.6	8.4	7.5	8.1
9	F.T.	23	M.	<	15.5	18.0	19.8	17.2	17.6	9.8	<	5.9	10.0	9.3	11.2	9.8	7.2

* < represents less than 2 mIU/ml

Fig. 3. Plasma LH and FSH responses to LH-RH in a patient with isolated gonadotropin deficiency (Case 8). Intravenous infusion of 400 μ g of LH-RH over a period of 2 hr were repeated on 7 successive days. The standard LH-RH test was performed before and on the 8th day of the LH-RH infusion.

One patient (Case 1) who exhibited subnormal response to 100 μ g of LH-RH showed a normal rise in plasma LH when 400 μ g of LH-RH was infused intravenously over a period of 2 hours. In two patients (Cases 3 and 4), the standard LH-RH test was repeated after 2 days treatment with intravenous infusion of 400 μ g LH-RH. However, plasma LH and FSH responses were not significantly affected. On the other hand, the responses to 100 μ g of LH-RH were significantly improved following treatment with LH-RH for 7 suc-

cessive days in 3 patients (Cases 7, 8, and 9) and for 21 successive days in one patient (Case 3). One of the representative data is shown in Figure 3. Details of the results will be published elsewhere (Yoshimoto Y., in preparation).

Plasma LH and FSH responses to LH-RH in patients with hypogonadism due to organic lesions in the hypothalamo-hypophyseal system (Table 2)

Basal plasma LH and FSH levels were

Table 2. Plasma LH and FSH responses to LH-RH in 8 patients with hypogonadism due to organic lesions in the hypothalamo-pituitary system.

Case	Name	Age	Sex	0	15	30	45	60	90	120	0	15	30	45	60	90	120
				LH						min.	FSH						min.
1	A.U.	19	F.	<*	<	5.5	<	<	<	4.0	<	<	<	<	<	2.6	<
2	O.Y.	17	M.	3.9	4.6	5.0	7.0	2.6	4.0	4.0	4.6	4.8	8.0	8.4	8.0	5.6	5.8
3	Y.A.	23	M.	<	4.3	4.4	4.8	4.4	4.3	<	<	2.2	<	<	<	<	<
4	M.M.	18	M.	2.5		6.6		13.4	11.0	<	<		<		<	<	4.8
5	Y.K.	15	M.	<	5.5	6.9		2.8	<	<	<	<	<		<	<	<
6	K.H.	17	M.	<	8.6	11.8	10.5	12.2	8.0	3.8	<	<	<	<	3.0	<	<
7	H.M.	26	M.	<		35.0		20.0	26.6	<	<		<		<	<	3.2
8	N.K.	23	M.	<	13.5	5.5	4.5	6.0	<	<	<	<	9.8	7.5	9.8	8.8	12.5

Case 1. Teratoma at the hypothalamus, Diabetes insipidus, post op. Case 2. Pituitary apoplexy, Diabetes insipidus Case 3. Pinealoma, Diabetes insipidus Case 4. Cured tbc meningitis Case 5. Cured tbc meningitis Case 6. Pinealoma, Diabetes insipidus Case 7. Short stature, Diabetes insipidus Case 8. Ectopic Pinealoma, Diabetes insipidus

* <represents less than 2 mIU/ml

undetectable in 6 and 7, respectively, out of 8 patients with failure of sexual maturation due to organic lesions in the hypothalamo-hypophyseal system. Plasma LH response to LH-RH was within normal limits in a patient, H. M., who had diabetes insipidus, short stature and poorly developed secondary sex characteristics of unknown etiology. The remaining 7 patients showed a significant but subnormal rise in plasma LH after the injection of LH-RH. In 2 of these patients, A. U. and O. Y., 400 μ g of LH-RH was given by a single intravenous injection without any improvement in plasma LH response. Plasma FSH responses to LH-RH were normal in two patients (O. Y. and N. K.), but virtually absent in the remaining 6 patients. In one patient with ectopic pinealoma (Y. A.), a possible hypothalamic lesion, the standard LH-RH test was repeated after treatment with intravenous infusion of 400 μ g of LH-RH for 7 successive days. As shown in Figure 4, plasma LH response to LH-RH, which was virtually absent before the treatment, was restored to normal after 7 day's treatment, with an increase in basal LH levels. Plasma FSH response to LH-RH was also normalized following the treatment with LH-RH.

Discussion

Measurement of plasma LH and FSH is a useful tool for the diagnosis of hypergonadotropic hypogonadism, because plasma levels of these hormones, especially FSH, are elevated in this disorder. However, plasma LH sometimes remains at high normal or slightly elevated levels. LH-RH test provides additional evidence for the diagnosis of this disorder, because plasma LH and FSH rose significantly from high basal levels as demonstrated in the present experiment. These results agree with those reported by Roth *et al.* (Roth *et al.*, 1972). On the other hand, measurement of basal plasma gonadotropin levels are less valuable for the diagnosis of gonadotropin deficiency, because there exists some overlap in plasma levels of LH and FSH between normal subjects and patients with gonadotropin deficiency (Odell *et al.*, 1967; Boyar *et al.*, 1973). This indicates a need for a simple and reliable method to evaluate pituitary gonadotropin reserve for the diagnosis of these patients. The present study demonstrates that plasma LH rose significantly and promptly in all normal subjects in response

Fig. 4. Plasma LH and FSH responses to LH-RH in a patient with hypogonadotropic hypogonadism due to ectopic pinealoma. Intravenous infusion of 400 μ g of LH-RH over a period of 2 hr were repeated on 7 successive days. The standard LH-RH test was performed before and on the 8th day of the LH-RH infusion.

to the intravenous injection of 100 μ g of LH-RH, while more than 200 μ g are necessary to obtain a consistent rise in plasma FSH. Furthermore, dose related responses of plasma LH and FSH were observed with LH-RH dose levels from 100 to 400 μ g. Aono *et al.* (Aono *et al.*, 1972) observed a dose-dependent rise in plasma LH following the administration of 5 to 400 μ g of LH-RH, although they failed to show a dose-response relationship in plasma

FSH responses. On the other hand, Yen *et al.* (Yen *et al.*, 1973) observed a dose-response relationship in plasma LH responses only between 25 to 150 μ g of LH-RH. These discrepancies may be explained at least in part by the different LH-RH preparations used in these experiments. Many investigators reported that 100 μ g of LH-RH provoked a sufficient response of plasma LH in all normal subjects tested (Abe *et al.*, 1972), whereas

plasma FSH response was not always consistent with this dose of LH-RH. These results may lead to the conclusion that a dose of 100 μ g is sufficient for evaluating LH reserve, while more than 200 μ g is preferable for evaluating FSH reserve.

In the present experiment, the standard LH-RH test was performed before and after treatment with intravenous infusion of 400 μ g of LH-RH for 2 to 21 days, in order to elucidate whether or not hyporesponsiveness of plasma LH can be restored by repeated stimulation with LH-RH. Two days treatment with LH-RH was not sufficient to restore plasma LH response to LH-RH in 2 patients with isolated gonadotropin deficiency. However, in 4 other patients, plasma LH response to LH-RH was significantly improved or completely normalized following 7 days treatment with LH-RH. In a female with familial gonadotropin deficiency, plasma LH response to LH-RH, which was absent before treatment, was completely normalized following treatment with LH-RH for 21 days. These results suggest that the hyporesponsiveness of plasma LH in patients with isolated gonadotropin deficiency results from prolonged understimulation by endogenous LH-RH and that the gonadotropin deficiency in this disorder is usually caused by a hypothalamic lesion. Two patients with organic hypopituitarism who showed limited response of plasma LH to LH-RH exhibited normal response following 7 days treatment with LH-RH.

These observations, in combination with those in isolated gonadotropin deficiency, indicate the need of repeated stimulation by LH-RH to differentiate hypothalamic from pituitary lesions.

References

- Abe, K., N. Nagata, S. Saito, K. Tanaka, T. Kaneko, N. Shimizu and N. Yanaihara (1972). *Endocrinol. Japon.* **19**, 77.
- Aono, T., J. Minamikawa, K. Kawamura, N. Terakawa, O. Tanisawa and K. Kurachi (1972). *Folia Endocr. Japon.* **48**, 578. (In Japanese)
- Boyar, R. M., J. W. Finkelstein, M. Witkin, S. Kapen, E. Weitzman, and L. Hellman (1973). *J. Clin. Endocrinol. Metab.* **36**, 64.
- Imura, H., Y. Yoshimoto and K. Moridera. *LH/FSH Releasing Hormone*, edited by Y. Yamamura, K. Kurachi and Y. Kumahara. Japanese Conference on LH/FSH Releasing Hormone, Tokyo, p 50 (1973).
- Odell, W. D., G. T. Ross and P. L. Rayford (1967). *J. Clin. Invest.* **46**, 248.
- Roth J. C., R. P. Kelch, S. L. Kaplan, and M. M. Grumbach (1972). *J. Clin. Endocrinol. Metab.* **35**, 926.
- Yen, S. S. C., R. Rebar, G. VandenBerg, F. Naftolin, H. Judd, Y. Ehara, K. J. Ryan, J. Rivier, M. Amoss, and R. Guillemin. *Hypothalamic Hypophysiotropic Hormones*, Excerpta Medica Foundation, p 217 (1973).