Biological and Immunological Characterization of Human Luteinizing Hormone Discharged by the Stimulation of Synthetic Luteinizing Hormone-Releasing Hormone (LH-RH) in Normal and Anovulatory Women

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Abstract

Plasma samples were obtained by repeated venopunctures immediately before, and at 15, 30, 60, 120 and 180 min after intravenous bolus administration of 100 μ g synthetic LH-RH in normal and various anovulatory women. Plasma hLH levels were determined by an *in vitro* bioassay and a radioimmunoassay with improved reagents. The LH-RH stimulation induced an abrupt elevation of both biological and immunological hLH activities in normal and anovulatory women, although the responsiveness to LH-RH differed from case to case. Both elevated biological and immunological hLH activities decreased gradually with a half disappearance time of 122.4 ± 27.9 min and 112.5 ± 25.4 min, respectively (Mean $\pm$ S.D.). A transient and significant depression in the ratio of biological to immunological hLH activities (B/I ratio) was observed at 15 min after the LH-RH administration in normal subjects. This depression is attributable to the cross-contamination of an increased amount of hLH subunits induced by LH-RH stimulation. The B/I ratios were significantly elevated throughout the investigation period in the anovulatory patients when compared with those in the normal subjects. This elevation appears to indicate the increased discharge of special type(s) of hLH subpopulations of high biological potency in the anovulatory cases.

A decapeptide with a potent action on the release of pituitary gonadotropins (luteinizing hormone-releasing hormone; LH-RH) has been synthesized and widely applied for various clinical and research purposes. It is evident now that the administration of synthetic LH-RH evokes a rapid and significant discharge of human luteinizing hormone (hLH) in human adults of both sexes.

It is rather well known that the LH is not a hormone of homology but consists of multiple subpopulations separable by isoelectrofocusing techniques (Robertson & Diczfalussy 1977; Wakabayashi, 1977; Ro-

bertson *et al.*, 1977; Van Damme *et al.*, 1977; Strollo *et al.*, 1981; Yano *et al.*, 1981; Zaidi *et al.*, 1982). Stimulation by LH-RH causes relative changes in the amount of hLH subpopulations discharged among subjects investigated; *i.e.* the increase in more acidic or neutral fractions was observed when postmenopausal plasma was compared with midcycle plasma (Strollo *et al.*, 1981) and when luteal plasma was compared with midcycle plasma (Yano *et al.*, 1981). Each hLH subpopulation appears to have its own biological and immunological properties (Yano *et al.*, 1981).

The purpose of the present study was to characterize the biological and immunological properties of hLH discharged by

LH-RH stimulation in normal and various anovulatory women.

Materials and Methods

Clinical material

Seven adult female volunteers participated in the present study. All of them were of fertile age (21–27 yr) with a history of regular cycles and had no medication. The LH-RH administration and blood sampling was performed in the follicular phase (cycle date of 6–10).

21 anovulatory patients were also investigated. 7 of these 21 patients were diagnosed as polycystic ovar syndrome (PCO) retrospectively with laparotomy and/or laparoscopic findings. The remaining 14 patients were classified into 3 groups in terms of hLH levels before LH-RH stimulation, *i.e.* hypergonadotropic (3 cases), normogonadotropic (9 cases), and hypogonadotropic (2 cases). The data obtained from 3 hypergonadotropic and 2 hypogonadotropic patients were discarded from statistical analysis owing to the paucity of the number.

To these normal volunteers and patients, 100 μ g synthetic LH-RH (Tanabe Pharmaceutical Co., Osaka, Japan) was administered intravenously at 9:00 AM. Blood was withdrawn by repeated venoduncture immediately before, and at 15, 30, 60, 120, and 180 min after the LH-RH administration. Plasma samples thus obtained were stored frozen at -70°C in aliquots until being assayed.

A plasma pool from multiple postmenopausal women was prepared for a quality control of the assays.

In vitro bioassay for hLH

The *in vitro* bioassay for hLH employed (Suginami *et al.*, 1981b) was a modification of that reported by Van Damme *et al.*, (1974). The method is based on the testosterone production by dispersed mouse testis interstitial cell preparations which is dependent on the doses of hLH. The method has been validated in its use on human female plasma (Romani *et al.*, 1977; Suginami *et al.*, 1981 a, b) and also on male plasma (Rajalakshmi *et al.*, 1979; Bartfai *et al.*, 1979). The mice used as the source of testis interstitial cells were derived from the C57 black strain aged 7 to 9 weeks (Suginami *et al.*, 1981b). All the samples were pre-treated with an equal volume of 0.5% Norit A charcoal for 30 min at 4°C with occasional agitation prior to centrifugation in order to remove substances of low molecular weight including various steroidal precursors (Rajalakshmi *et al.*, 1979). This modification was introduced in the present study as a precautionary step rather than out of necessity. The standard

preparation employed was the human pituitary LH 1st IRP for immunoassay (Code No. 68/40), supplied by the National Institute for Biological Standards and Control, London, England. The sensitivity of the assay was 6.25–12.5 μ IU/tube. The index of precision (λ) was 0.02–0.05. The quality control plasma (the pooled postmenopausal plasma) was measured by this method and found to be 88.3 ± 5.8 mIU/ml (Mean $\pm$ S.D.; $n=11$).

hLH radioimmunoassay

An equilibrium radioimmunoassay (RIA) method was employed in the assessment of immunological activities of hLH with improved reagents (Suginami *et al.*, 1978, 1981a). An anti-hLH serum and a highly purified human pituitary LH preparation (LER 960) were provided for the present study by the National Pituitary Agency, NIH, Bethesda, Maryland, USA. The LER 960 preparation was iodinated with a lactoperoxidase method (Suginami *et al.*, 1981a). The iodinated products were purified by gel filtration on Ultrogel AcA 54 (IKB Produkter AB, Bromma, Sweden) as described by Suginami *et al.* (1978). The incubation of sample plasma or the 68/40 preparation (200 μ l) for 3 days at room temperature with iodinated hLH (100 μ l; *ca.* 20,000 dpm) and anti-hLH serum (100 μ l; diluted 1:8000) was terminated by the double antibody procedure. The sensitivity of the assay was 0.625–1.25 mIU/tube. The index of precision (λ) was 0.02–0.05. The same quality control plasma as was also used in the *in vitro* bioassay was measured by this method and found to be 67.9 ± 5.3 mIU/ml (Mean $\pm$ S.D.; $n=7$).

Assay design

All the assay tubes were randomized in order to eliminate systematic errors. A multiple 2+2 parallel line assay design (Finney 1978) was employed with 2 dose levels and 3 replicates per dose level in the assessment of the hLH activities of the sample plasma after the estimation of parallelism against the linearized standard response line in both the bioassay and RIA. A logit log dose transformation was employed for linearization in both assays (Robertson *et al.*, 1978; Suginami *et al.*, 1981a, b). Samples showing non-parallelism were omitted from further analyses, although most of them showed statistical parallelism in both assays.

The half disappearance time ($t_{1/2}$) of hLH from circulation was calculated by the linear regression lines with the estimates at 30, 60, 120 and 180 min after the LH-RH administration after logarithmic transformation. Some of the statistical methods, such as two way analysis of variance, Student's *t*-test, Welch's *t*-test, *etc.*, were employed for the evaluation of the results obtained.

Results

Normal subjects

Plasma levels of biological and immunological hLH activities before the LH-RH administration (control values) were 14.7 ± 5.6 mIU/ml (Mean $\pm$ S.D.) and 14.1 ± 4.7 mIU/ml, respectively. Intravenous administration of 100 μ g synthetic LH-RH evoked an abrupt elevation of plasma levels of both biological and immunological hLH activities, reaching 80.3 ± 35.8 mIU/ml and 72.2 ± 28.3 mIU/ml, respectively, at 30 min after the LH-RH administration (Table 1). As time passed the elevated plasma hLH levels declined gradually (Table 1) and linearly when the estimates were plotted on a semi-logarithmic scale. The $t_{1/2}$'s of biological and immunological hLH activities were 125.8 ± 21.7 min and 106.2 ± 19.7 min, respectively, showing no statistical significance (Table 3). The ratios of biological to im-

munological hLH activities (B/I ratios) were maintained above unity throughout most of the treatment period, although a significant depression was observed at 15 min after the LH-RH administration (0.94 ± 0.09 ; $p < 0.05$) (Table 2).

Table 3. The half disappearance time of biological and immunological activities of hLH discharged by the intravenous administration of 100 μ g synthetic LH-RH in normal follicular phase (n=7), PCO (n=7) and normogonadotropic anovulatory patients (n=9). Means and standard deviations are presented.

	Half disappearance time (min)	
	Bioactivity	Immunoactivity
Normal	120.8 ± 21.7	106.2 ± 19.7
PCO	124.5 ± 22.3	112.8 ± 21.6
Anov.	123.6 ± 30.5	115.1 ± 28.1
Total	122.4 ± 27.9	112.5 ± 25.4

The half disappearance time was calculated by the linear regression lines with the estimates at 30, 60, 120 and 180 min after the LH-RH administration.

Table 1. Plasma hLH levels before and after the LH-RH stimulation in normal follicular phase (n=7), PCO (n=7) and normogonadotropic anovulatory patients (n=9) as measured by the bioassay and RIA. Means and standard deviations are presented.

		Time after LH-RH administration (min)					
		0	15	30	60	120	180
Bioassay	Normal	14.7 ± 5.6	55.5 ± 25.9	80.3 ± 35.8	69.3 ± 32.0	53.7 ± 20.6	40.2 ± 11.9
	PCO	52.5 ± 11.3	189.8 ± 30.5	242.6 ± 57.2	225.2 ± 44.2	155.4 ± 19.4	123.6 ± 17.7
	Anov.	23.3 ± 13.5	73.3 ± 25.3	112.0 ± 52.9	100.7 ± 46.9	71.0 ± 31.6	50.9 ± 27.0
RIA	Normal	14.1 ± 4.7	60.3 ± 28.5	72.2 ± 28.3	60.3 ± 24.8	47.7 ± 18.9	36.9 ± 9.6
	PCO	36.8 ± 6.7	140.8 ± 37.8	183.9 ± 50.4	145.8 ± 42.6	98.6 ± 29.9	82.6 ± 21.5
	Anov.	22.6 ± 7.2	67.0 ± 25.1	90.0 ± 40.6	70.6 ± 30.1	48.3 ± 15.4	37.5 ± 9.9

Results are expressed in terms of mIU/ml of the 68/40 standard preparation.

Table 2. The ratios of biological to immunological activities of hLH (B/I ratios) before and after the LH-RH stimulation in normal follicular phase (n=7), PCO (n=7) and normogonadotropic anovulatory patients (n=9). Means and standard deviations are presented.

		Time after LH-RH administration (min)					
		0	15	30	60	120	180
Normal		1.04 ± 0.07	$0.94 \pm 0.09^\dagger$	1.09 ± 0.13	1.13 ± 0.14	1.16 ± 0.20	1.23 ± 0.23
PCO		$1.44 \pm 0.25^{**}$	$1.41 \pm 0.33^*$	1.36 ± 0.29	$1.59 \pm 0.24^{**}$	$1.64 \pm 0.41^*$	1.55 ± 0.28
Anov.		$1.24 \pm 0.24^*$	$1.18 \pm 0.28^*$	1.34 ± 0.36	$1.51 \pm 0.37^*$	$1.53 \pm 0.39^*$	1.39 ± 0.36

* Statistical significance against the corresponding values obtained in the normal subjects ($p < 0.05$).

** Statistical significance against the corresponding values obtained in the normal subjects ($p < 0.01$).

† Statistical significance against the B/I ratios before the LH-RH stimulation ($p < 0.05$).

PCO cases

The control values for both biological and immunological hLH activities for these PCO patients (52.5 ± 11.3 mIU/ml and 36.8 ± 6.7 mIU/ml, respectively) were significantly higher than those for normal subjects (Table 1). The LH-RH stimulation induced also a prominent discharge of hLH, although the responsiveness to injected synthetic LH-RH (as reflected by the net increase of hLH activities) was much enhanced when compared to that in the normal subjects (Table 1). The $t_{1/2}$'s of biological and immunological hLH activities in these PCO patients were 124.5 ± 22.3 min and 112.8 ± 21.6 min, respectively. These two values were not significantly different from each other or from those in the normal subjects (Table 3). The B/I ratios in these patients were elevated significantly throughout the investigation period in comparison with the corresponding values in the normal subjects ($p < 0.01$ at Time 0 and 60, and $p < 0.05$ at Time 15 and 120) (Table 2).

Normogonadotropic anovulatory cases

The responsiveness, in terms of hLH discharge from the pituitary, to injected synthetic LH-RH in these patients was similar to that in the normal subjects (Table 1). The $t_{1/2}$'s of elevated biological and immunological hLH activities were comparable to each other and not different from those in the normal subjects (Table 3). However, the B/I ratios in these patients were elevated significantly throughout the investigation period when compared with the corresponding B/I ratios in the normal subjects ($p < 0.05$ at Time 0, 15, 60 and 120) (Table 2),

Discussion

A significant elevation of plasma hLH levels was detected by both the *in vitro* bioassay and RIA employed in the present

study when 100 μ g synthetic LH-RH was administered intravenously to normal and various anovulatory women. These two assays provided comparable data to those reported by many investigators as far as the profile of hLH levels is concerned.

The elevated plasma hLH levels decreased gradually and linearly when the estimates obtained were plotted on a semi-logarithmic scale. The $t_{1/2}$'s of both biological and immunological hLH activities in the anovulatory patients were not different from those in the normal subjects. These $t_{1/2}$ values are comparable to those obtained on naturally occurring hLH pulses in the normal menstrual cycle (Santen & Bardin, 1973; Suginami *et al.*, 1982). Furthermore, they are in close agreement with the results in the literature (Schalch *et al.*, 1968; Kohler *et al.*, 1968; Nankin & Troen, 1972; Yen *et al.*, 1972) and significantly longer than the rapid half life of endogenous hLH after complete hypophysectomy (Yen *et al.*, 1968). This difference could be attributed to the residual action of LH-RH upon intact pituitary.

A significant and transient depression in B/I ratios soon after the LH-RH administration was observed in the normal subjects in the present study. This depression is attributable to the cross-contamination of an increased amount of hLH subunits, positive immunological activity and lack of biological activity in plasma under the influence of the administered synthetic LH-RH (Benveniste *et al.*, 1973, 1975; Hagen & McNeilly, 1975a, b). Most of the hLH subunits secreted by LH-RH stimulation are likely to disappear from circulation in a short time course (Braunstein *et al.*, 1972; Benveniste *et al.*, 1975). Because of the rapid disappearance of hLH subunits, the depressed B/I ratio could not have lasted long and was recovered soon. In contrast with the results obtained in the present study, the absence of the depression of B/I ratios after LH-RH stimulation was reported

in normal subjects (Dufau *et al.*, 1976). This disagreement seems to be owing to the methodological differences or to the paucity of the number of subjects examined in their study.

One of the most important items of evidence obtained in the present study is that the B/I ratios in the anovulatory patients were significantly elevated throughout the investigation period in comparison with those in the normal subjects. This is interpreted as indicating that the special type(s) of hLH subpopulations of relatively high biological potency is secreted more in these anovulatory patients than in the normal subjects. It is now quite well known that the LH is not an homologous hormone but consists of multiple subpopulations separable by isoelectrofocusing techniques (Robertson & Diczfalusy, 1977; Wakabayashi 1977; Robertson *et al.*, 1977; Van Damme *et al.*, 1977; Strollo *et al.*, 1981; Yano *et al.*, 1981; Zaidi *et al.*, 1982). When pituitary homogenates, plasma or urinary samples are fractionated by isoelectrofocusing techniques, the majority of LH activity migrates to the alkaline region. Recent investigations demonstrated relative changes in the amount of hLH subpopulations under various conditions. For instance, the increase in acidic or neutral fractions was observed when postmenopausal plasma was compared with midcycle plasma (Strollo *et al.*, 1981) and when female plasma in the luteal phase was compared with midcycle plasma (Yano *et al.*, 1981). Each hLH subpopulation appears to possess its own biological and immunological properties; *i.e.* the B/I ratio of acidic hLH is significantly and markedly decreased when compared with that of alkaline hLH (Robertson & Diczfalusy, 1977; Robertson *et al.*, 1977; Yano *et al.*, 1981). Identification of the hLH subpopulation(s) which caused the elevation of B/I ratios in the anovulatory patients in the present study is to be performed in future.

Acknowledgements

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