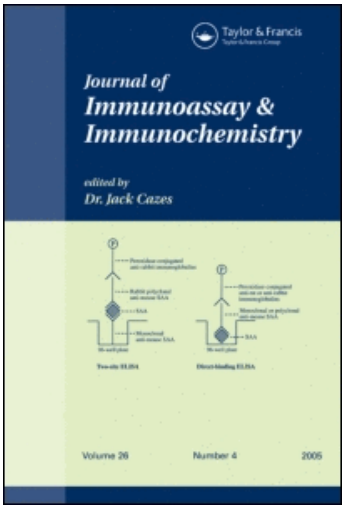

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ALPHA AND BETA SUBUNIT IMMUNOREACTIVITY OF REFERENCE PREPARATIONS USED IN THE RADIOIMMUNOASSAY OF FOLLICLE STIMULATING AND LUTEINIZING HORMONES IN SERUM

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ABSTRACT

The 2nd IRP-HMG, the 1st IS, and LER 907 have been widely used in standard FSH and LH RIA systems to measure circulating levels of FSH and LH in human serum. In these systems, the dose-response curves elicited by the preparations were parallel to each other and to that of serum samples tested. When these materials were studied in the homologous α and β-hLH RIA systems, the three hormones and a serum sample used as internal control in all assays, displayed a higher degree of contamination with α than with β-hLH subunits. On weight basis, the α/β ratios were 18.0, 18.7, 6.7, and 13.0, for the 2nd IRP-HMG, 1st IS, LER 907 and serum, respectively. In the homologous α and β-hFSH RIA's, the highest contamination with α-hFSH was displayed by LER 907 (α/β ratio:10), the α/β ratios for the 2nd IRP-HMG and 1st IS were 2.2 and 1.1, respectively. The serum sample contained about 6.2 ng equivalent of α-hFSH/ml; β-hFSH was not detected.

The α-subunits of glycoprotein hormones are interchangeable and responsible for the "within species" cross-reactivity of the hormones. Therefore, employing an antisera raised against native FSH and LH in which the antigenic sites are α-directed, the reference materials and the circulating gonadotropins which contain an excess of α over β-subunit, would elicit dose-response curves parallel to each other. Because various batches of RIA reagents may not exhibit similar characteristics, before these materials are used in standard RIA's, the immunological similarity of the reference preparation and test samples must be established with the reagents used in each laboratory.

INTRODUCTION

The 2nd IRP-HMG and the 1st IS, both of urinary origin, and the pituitary extract, LER 907, commonly used as reference preparations in the radioimmunoassay (RIA) of follicle-stimulating and luteinizing hormone in serum, are not alike. Not only have they been extracted from different sources but, they differ in the degree of homogeneity, and in physicochemical properties, and may contain a variety of partly degraded materials which may be distinguishable in some, but not all RIA systems. In spite of these differences, the slope of the inhibition lines elicited by these preparations were parallel to each other in the homologous FSH and LH standard RIA systems (1). In order to gain an understanding regarding these findings, studies were carried out testing these preparations in the homologous alpha and beta hFSH and hLH RIA subunit systems.

MATERIALS AND METHODS

Appropriate stocks and substock solutions of the three hormones were prepared as previously reported (1). The characteristics of the alpha and beta RIA's of hFSH and hLH performed under our laboratory conditions have been previously described (2). The double antibody RIA method was used, employing reagents supplied by the National Pituitary Agency (NPA), Baltimore, Maryland. In each of the four RIA systems, the respective subunit was used as label and as the reference preparation.

The minimum detectable dose (MDD) of the reference preparation used was defined as the dose which produced 80% of the binding of label by the zero standard. In the α and β-hFSH systems MDD's were 0.82 ng for α-hFSH (NPA Batch 1), and 0.29 ng for β-hFSH (NPA Batch 1), respectively; the interassay coefficient of variation of replicate assays was 0.06 and 0.07, respectively. In the α and β-hLH systems the MDD's were 0.07 ng for α-hLH (NPA Batch 1), and 0.025 ng for β-hLH (NPA Batch 1), respectively; the interassay coefficient of variation of replicate assays was 0.07 and 0.18, respectively.

One IU of FSH and LH activity of the 2nd IRP-HMG was considered to be contained in 229.5 mcg of the material (WHO, Technical Report Series 293, 1964), and 1 IU of FSH and of LH activity of the 1st IS, was considered to be contained in 133.9 and 133.7 mcgs of this standard, respectively (3). The following dose levels were used: in the α-hFSH subunit system, α-hFSH (reference preparation), from 1 to 5 ng; β-hFSH, from 10 to 100 ng; 2nd IRP-HMG, 1.1, 1.7, 2.3, 4.6 and 9.2 mcg; 1st IS, 1.0, 1.3, 2.7 and 5.4 mcg; and 30, 50, 150 and 200 ng of LER 907. In the β-hFSH subunit system: β-hFSH (reference preparation), from 0.2 to 10 ng; α-hFSH, from 100 to 300 ng; 2nd IRP-HMG, 2.2, 4.6 and 9.2 mcg; 1st IS, 0.67, 1.0, 1.3, 2.7 and 5.4 mcg; and 100, 200 and 400 ng of LER 907; in the α-hLH subunit system; α-hLH (reference preparation), from 0.05 to 1 ng; β-hLH; from 40 to 400 ng; 2nd IRP-HMG, 0.46, 0.69, 1.1, 1.7 and 2.3 mcg; 1st IS, 0.4, 0.67, 1.0, 1.3 and 2.7 mcg; and 5,10,20 ng of LER 907. In

the β-hLH subunit system: β-hLH (reference preparation) from 0.05 to 1 ng; α-hLH, from 5 to 100 ng; 2nd IRP-HMG, 2.3, 4.6 and 6.9 mcg; 1st IS, 1.4, 2.7, 4.0 and 5.3 mcg; and 10, 20, 50 and 100 ng of LER 907. Three dilutions of an unfractionated serum sample obtained from several normal men, used as internal control in our laboratories, were tested simultaneously with the preparations under study. All assays were calculated by appropriate computer programs. For 2nd IRP-HMG and 1st IS, results are expressed both in terms of mass and of the corresponding IU's, and, in terms of mass for LER 907.

RESULTS

Table 1 depicts the results obtained when the preparations were studied in the α and β-hFSH RIA systems. The contamination of α-hFSH with β-hFSH was 0.4%, and that of β-hFSH with α-hFSH 7%. The three hormones displayed a higher degree of contamination with α than with β-hFSH. On weight basis, the α/β ratios were: 2.7, 1.1 and 10.0 for the 2nd IRP-HMG, 1st IS and LER 907, respectively. α-hFSH was measured in the serum sample, however, due to the lower sensitivity of the β-hFSH system, β-hFSH was undetectable.

Table 2 depicts the results obtained in the α and β-hLH RIA systems. The contamination of α-hLH with β-hLH was 0.8%, and that of β-hLH with α-hLH 0.1%. The three hormones displayed a higher degree of contamination with α than with β-hLH. On weight basis, the α/β ratios were 18.0, 18.7 and 6.7 for the 2nd IRP-HMG, 1st IS and LER 907, respectively. The serum sample

TABLE 1

RIA's of 2nd IRP, 1st IS and LER 907 in the α and β-hFSH Subunit System

ONS	α-hFSH RIA SYSTEM			β-hFSH RIA SYSTEM	
	RELATIVE POTENCY (CONFIDENCE LIMITS)	SLOPE	MED +	RELATIVE POTENCY (CONFIDENCE LIMITS)	SLOPE
	1	-1.1	1.7 ng	0.004 (0.002-0.006) ng/ng	-1.1
	0.07 (0.05-0.08) ng/ng	-1.2	25.3 ng	1	-0.9
	0.00041 ng/ng (0.00035-0.00047) [100 80-110) ng/IU]	-1.1	4.2 mcg [18.1 mIU]	0.00015 ng/ng (0.0001-0.00023) [40 (30-60) ng/IU]	-1.0
	0.00032 ng/ng (0.00024-0.0004) [40 (33-60) ng/IU]	-1.1	5.3 mcg [39.7 mIU]	0.00029 ng/ng (0.0002-0.0004) [49 (37-64) ng/IU]	-1.0
	0.03 (0.025-0.04) ng/ng	-1.1	55.6 ng	0.003 (0.002-0.004) ng/ng	-1.2
trol	6.2 (2.9-9.6) ng/ml	-1.2	367.4 λ	*	

ian Effective Dose (50% binding). *Undetectable. Relative Potency: mean of 2 assays.

TABLE 2

RIA's of 2nd IRP, 1st IS and LER 907 in the α and β-hLH Subunit System

PREPARATIONS	α-hLH RIA SYSTEM			β-hLH RIA SYSTEM		
	RELATIVE POTENCY (CONFIDENCE LIMITS)	SLOPE	MED +	RELATIVE POTENCY (CONFIDENCE LIMITS)	SLOPE	MED +
α-hLH	1	-1.3	0.18 ng	0.008 (0.006-0.011) ng/ng	-0.6 *	16.0 ng
β-hLH	0.001 (0.0007-0.0015) ng/ng	-1.3	148.0 ng	1	-1.1	0.14 ng
2nd IRP HMG	0.00027 ng/ng (0.00018-0.00044) [64 (49-88) ng/IU]	-1.1	0.55 mcg [2.4 mIU]	0.000015 ng/ng (0.000012-0.00002) [3.6 (2.5-5.0) ng/IU]	-0.9	9.1 mcg [39.4 mIU]
1st IS	0.00028 ng/ng (0.00018-0.00046) [39 (29-55) ng/IU]	-1.1	0.52 mcg [3.9 mIU]	0.000015 ng/IU (0.000011-0.000021) [2.2 (1.4-3.4) ng/IU]	-0.9	8.4 mcg [63.2 mIU]
LER 907	0.02 (0.01-0.03) ng/ng	-1.2	7.8 ng	0.003 (0.002-0.004) ng/ng	-1.0	54.0 ng
Serum Control	3.1 (2.2-2.4) ng/ml	-1.2	82.0 λ	0.24 (0.12-0.4) ng/ml	-1.2	434.0 λ

+MED: Median Effective Dose (50% binding). *Non-parallel (F significant at 0.05 level). Relative Potency: mean of 2 assays.

showed a higher content of α-hLH than of β-hLH (α/β ratio:13). In each of the four RIA systems, the slopes of the inhibition lines elicited by all preparations except for that of α-hLH in the β-hLH system (Table 2), were parallel to the respective reference material and to each other (F not significant at 0.05 level).

DISCUSSION

The radioimmunoassays of FSH and LH were first developed in 1966 and have since been widely used to perform studies related to gonadotropic secretion in physiological and pathological conditions in men. These studies have been, and are performed examining the circulating levels of these hormones employing antisera raised against the native hormones. FSH and LH are present in tissues, plasma or urine in heterogenous forms. Plasma or serum may contain not only the native hormones but some of their metabolic products. Also, free α-subunits of hFSH, hLH and hTSH have been found in serum (2, 4 to 7), and circulating free β-hLH subunits have been measured throughout the menstrual cycle in women (2).

Because of the presence of common antigenic sites between glycoprotein hormones, antisera raised against one of the gonadotropins may contain antibodies capable of reacting indistinctly with all the glycoprotein hormones. Therefore, it is difficult to determine whether the cross-reaction observed when employing antisera raised against native glycoprotein hormones reflects contamination of hormonal preparations used

as immunogens or, alternatively, reflect the presence of common antigenic determinants in small portions or entire subunits of the hormones.

The choice of reference preparations poses difficulty in the general application of standard RIA's for the measurement of gonadotropins in blood. One of the fundamental principles of all bioassays or immunoassays is that the reference preparation or standard used be as similar as possible to the material being tested. At present, preparations suitable for the RIA estimation of serum FSH and LH are not available and materials such as the 2nd IRP-HMG, the 1st IS, and LER 907 are widely used for this purpose.

The present studies have demonstrated the heterogeneity of these hormones. When studied in the α and β-hLH RIA's, they elicited higher contamination with α than with β-subunits. In the 2nd IRP-HMG and the 1st IS, the contamination with α-hLH was three times higher than in LER 907. In the α and β-hFSH RIA's, the highest contamination with α-hFSH was elicited by LER 907 (α/β:10), the 2nd IRP-HMG and the 1st IS contained two times or equal the amount of α than of β-FSH, respectively. In the serum sample, α-hFSH was measured; β-hFSH was undetectable. Studies by other investigators have shown that after gel filtration in Sephadex G-100, the immunoreactive elution patterns of the 2nd IRP-HMG, and of LER 907 indicated distinct immunoreactive peaks of FSH and LH, as well as of free α-subunits of FSH and LH. Free β-FSH was not detected, and a

well defined reactive peak of free β-hLH was not seen (8,9,10).

It has been well established that all four glycoprotein hormones contain two noncovalently bound subunits, α and β (11-16). Separately, neither subunit exerts biologic activity. The β-subunits confer biologic and immunologic specificity and are responsible for the "between species" cross-reactivity of the glycoprotein hormones; the α-subunits are sufficiently similar to be interchangeable with one another, and are responsible for the "within species" cross-reactivity of the hormones (17,18). Therefore, employing an antisera raised against native FSH and LH, in which the antigenic sites are α-directed, the slopes of the inhibition lines elicited by urinary preparations such as the 2nd IRP-HMG and 1st IS, by a pituitary extract such as LER 907, and by human serum,all of which contain an excess of α over β-subunits, would be parallel to each other.

Certainly, a reference preparation or standard cannot be used unless it gives parallelism with the test sample in the assay system utilized. It should be remembered, however, that parallelism in itself, does not provide proof that the substances in the test serum and the reference material or materials are identical, or that they are appropriate for use in another assay system or, more importantly, that they are appropriate for use with other batches of reagents in similar assay systems, as replacement reagents may differ as far as their immunological characteristics, specificity and sensitivity are concerned. Consequently, before the 2nd IRP-HMG, the 1st IS or LER 907

are used as reference preparations, their immunological similarity with the test samples must be established with the RIA reagents used in each laboratory.

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