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Application of Ovine Luteinizing Hormone (LH) Radioimmunoassay in the Quantitation of LH in Different Mammalian Species

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ABSTRACT. A sensitive double antibody radioimmunoassay has been developed for measuring luteinizing hormone (LH) in various African mammalian species, using rabbit anti-ovine LH serum (GDN 15) and radioiodinated rat LH or ovine LH. Serum and pituitary homogenates from some African mammals (hyrax, reedbuck, sable, impala, tsessebe, thar, spring-hare, ground squirrel and cheetah, as well as the domestic sheep, cow and horse and laboratory rat and hamster) produced displacement curves parallel to that of the ovine LH standards. The specificity of the assay was examined in detail for one species, the rock hyrax. Radioimmunoassay and bioassay estimates of LH in hyrax pituitaries containing widely differing quantities of pituitary hormones were similar. In sexually active male hyrax mean plasma LH was 12.1 ng/ml and pituitary LH 194 μg/gland, but in sexually quiescent hyrax mean plasma LH was 2.4 ng/ml and mean pituitary LH 76 μg/gland. Intravenous injection of 10 μg of luteinizing hormone releasing hormone increased mean LH levels in hyrax from 0.9 ng/ml to 23.2 ng/ml by 30 min. Conversely, im injection of 250 μg testosterone induced a fall in LH levels in male hyrax from 1.7 ng/ml to 0.7 ng/ml 6 h after administration. Although the specificity of the assay for quantitating plasma LH in other species was not categorically established, there was a good correlation between plasma LH concentration and reproductive state in the bontebok, impala, spring-hare, thar, cheetah, domestic horse and laboratory rat, suggesting the potential use of the antiserum in quantitating LH in a variety of mammalian species. (*Endocrinology* 101: 760, 1977)

STUDIES on the reproductive physiology of wild mammals, and in particular, seasonal breeders, have contributed to a more complete understanding of the endocrine mechanisms controlling reproductive processes in general. Although it has been possible to measure pituitary levels of gonadotrophic hormones in wild animals by bioassays (1,2), the inability to assay circulating levels of these hormones has hampered advances in the field.

Subsequent to the development of radioimmunoassays for measuring luteinizing hormone (LH) in man (3), rat (4) and sheep (5), heterologous radioimmunoassays employing antisera raised against LH from a different species have been successfully used to measure LH in plasma of several species including the rat (6), hamster (7), gerbil (8), rabbit (9) and vole (10). It seemed feasible, therefore, that heterologous LH radioimmunoassay might allow the measurement of LH in wild African mammals.

This investigation describes the development, validation, and application of a heterologous radioimmunoassay for determining plasma LH in African mammals.

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Materials and Methods

Antisera and LH preparations

An antiserum to rat LH (NIAMDD-A-rat LH-S-1) and one raised against ovine LH (Dr. G. D. Niswender—GDN 15) were initially tested. The ovine LH antiserum was more suitable and subsequently used exclusively. Purified LH preparations for radioiodination were rat LH (NIAMD-rat LH-I-3), ovine LH (LER-1056-C2), and bovine LH (W. Carr CG-3). Ovine LH (NIH-LH-S18) was used as standard. This preparation has an LH potency of 1.03 times NIH-LH-S1/mg with an FSH contamination of less than 0.05 NIH-FSH-S1 units/mg. Other preparations used included human growth hormone (hGH), rat growth hormone (rGH), human chorionic gonadotropin (hCG), human menopausal gonadotropin (hMG), human thyroid stimulating hormone (hTSH), avian LH (Ball AE1/B2) and ovine follicle stimulating hormone (NIH-FSH-S10) which has an LH contamination of approximately 0.01 NIN-LH-S1 units/mg.

Radioiodination

Purified LH preparations were radioiodinated using a modification of the method of Greenwood, Hunter and Glover (11). Twenty-five μl of 0.5M sodium phosphate buffer (pH 7.6) was mixed with 2 μg of LH (20 μl). Immediately after addition of 1 mCi Na ^{125}I (Radiochemical Centre, Amersham), 50 μg of chloramine T (10 μl) was added and the mixture agitated for 1 min. The reaction was stopped by addition of 250 μg metabisulfite in 20 μl phosphate buffer. Separation of radioiodinated LH from unreacted Na ^{125}I in the mixture was accomplished by elution with 0.5M phosphate buffer containing 1% bovine serum albumin (BSA) on a Sephadex G-50 column (15 × 0.5 cm). In an alternative method, [^{125}I]iodo-LH was separated by adsorption chromatography on a Whatman CF 11 column (0.6 × 1.5 cm) (12). After applying the mixture, free Na ^{125}I was eluted from the column with 40 ml of 0.12M barbiturate buffer (pH 8.6) and the [^{125}I]iodo-LH then eluted with 10 ml human serum. To determine the percentage of ^{125}I incorporated into LH and the effectiveness of purification procedures, aliquots were diluted in 0.12M barbiturate buffer (pH 8.6) and subjected to electrophoresis on Toyo 514 paper or Oxoid cellulose acetate paper.

Radioimmunoassay procedure

The radioimmunoassay was essentially that described by Niswender *et al.* (6). Various incubation conditions at 25 C or 4 C were tested. These included simultaneous addition of [^{125}I]-iodo-LH and LH standard, preincubation of standard LH before addition of [^{125}I]iodo-LH and incubation periods varying from 5 h to 3 days. The assay system finally adopted involved a preincubation of standards or test samples with antibody for 24 h followed by the addition of [^{125}I]iodo-LH and incubation for 6 h at 25 C. Antibody-bound [^{125}I]iodo-LH was then precipitated by incubating with anti-rabbit gamma-globulin overnight at 4 C and centrifugation.

Animal material

Blood was collected in heparinized tubes from captive animals and various species of wild mammals which were immobilized, or shot in culling programs. The blood was immediately centrifuged and the plasma separated and stored at −20 C. Whenever possible the anterior pituitary was also collected, frozen and later homogenized in 2 ml ice cold PBS (Potter homogenizer), centrifuged at 40,000 × *g* for 30 min and the supernatant diluted appropriately for assay. Specimens included plasma from male and female adults in various reproductive states as well as immature animals. The species studied were: the cheetah (*Acinonyx jubatus*), spotted hyena (*Crocuta crocuta*), spring-hare (*Pedetes capensis*), porcupine (*Hystrix africae-australis capensis*), ground squirrel (*Xerus inauris*), bontebok (*Damaliscus dorcas dorcas*), blesbok (*Damaliscus dorcas phillipsi*), reedbuck (*Redunca arundinum*), sable (*Hippotragus niger*), roan antelope (*Hippotragus equinus*), impala (*Aepyceros melampus*), tsessebe (*Damaliscus lunatus*), springbok (*Antidorcas marsupialis*), thar (*Hemitragus jemlahicus*), bush baby (*Galago crassicaudatus*), rhinoceros (*Cerathotherium simum*), and rock hyrax (*Procavia capensis*).

In order to validate the assay for LH in the rock hyrax, LH immunoreactivity and biological activity were compared in pituitary samples with different relative content of other pituitary protein hormones. In addition, plasma LH was quantitated before and after iv administration of 10 μg luteinizing hormone releasing hormone (LHRH), 100 μg thyrotropin releasing hormone (TRH) and im injection of 250 μg testosterone. Male impala were given 100 μg LHRH iv.

Plasma LH was determined during the estrous cycle of the mare and also in normal adult male rats, rats castrated four weeks previously and male rats which had received 10 mg estradiol implants ten weeks previously.

Plasma testosterone concentration in male animals was determined by conventional radioimmunoassay utilizing a highly specific antiserum, raised against testosterone-3-carboxymethyl oxime conjugated to haemocyanin, which exhibited only 5% cross-reaction with dihydrotestosterone (Millar and Kewley, unpublished).

Biological assays

LH activity in hyrax anterior pituitaries was determined by the ovarian ascorbic acid depletion (OAAD) bioassay (13) using NIH-LH-S18 as standard preparation. Follicle stimulating hormone (FSH) activity was determined by the hCG-

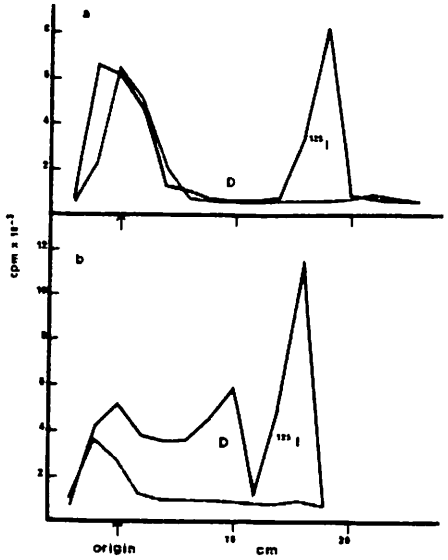

FIG. 1. Electrophoretograms of iodination reaction mixtures and purified [^{125}I]iodo-LH. (*a*) Reaction mixture (——) after iodinating with 25 μg chloramine T, and fraction 7 (– – –) from the Sephadex G-50 column. (*b*) Reaction mixture (——) after iodinating with 50 μg chloramine T, and [^{125}I]iodo-LH eluted from CF 11 cellulose column with human plasma (– – –). Both purification methods illustrate that damaged hormone (D) and free iodide [^{125}I] have been removed leaving only [^{125}I]iodo-LH at the origin of the electrophoretogram.

augmentation method (14) using NIH-FSH-S11 as standard.

Results

Radioiodination

Sephadex G-50 columns yielded a good separation of [^{125}I]iodo-LH from free Na ^{125}I. The electrophoretogram (Fig. 1a) demonstrates that material in the seventh 1 ml fraction from the G-50 column is essentially free of Na ^{125}I. The Whatman CF 11 column also satisfactorily removed Na ^{125}I and in addition was more efficient in removing the increased amount of "damaged" hormone which was invariably produced when large amounts of chloramine T were used to obtain higher specific activity [^{125}I]iodo-LH (Fig. 1b). The specific activity of radioiodinated LH when using 50 μg chloramine T was much higher than that achieved with 25 μg and in the particular iodination depicted was 316 μCi/μg. Since the Sephadex G-50 did not remove "damaged" hormone as efficiently as CF 11, the latter was used when purifying material after iodinating in the presence of 50 μg chloramine T. [^{125}I]Iodo-LH prepared in this way could be successfully used in assays for at least 14 days. Immunoreactivity did not decrease severely. For example, in a preparation in which 32% of [^{125}I]iodo-LH was bound in the assay system on the first day, 29% was bound after 4 days storage at 4 C, 23% after 9 days and 19% after 14 days. The sensitivity of the assay (taken as the minimum amount of LH which significantly inhibited binding of [^{125}I]iodo-LH) was only slightly decreased with storage for 14 days and the standard curves were virtually superimposable. [^{125}I]-Iodo-LH eluted from the CF 11 column could be successfully stored at −20 C for at least one month.

Radioimmunoassays

In studies using rat [^{125}I]iodo-LH, the assay employing conditions described by Niswender *et al.* (6) conducted at 4 C required the least mass of standard LH to cause displacement of 50% of the [^{125}I]iodo-LH. Of the various assay systems tested at 25 C, the greatest sensitivity was achieved by utilizing a non-equilibrium incubation for 6 h (see Fig. 2). These conditions were used in the studies on LH in pituitary samples (Fig. 4). Inclusion of 100 μl human plasma in assay tubes produced a slight displacement of the standard curve (Fig. 2), but no further displacement occurred when 200 μl or 300 μl plasma was used. This nonspecific displacement only occurred with certain individual plasma samples (compare human plasma in Fig. 5 which had no effect) and it was therefore thought not worthwhile

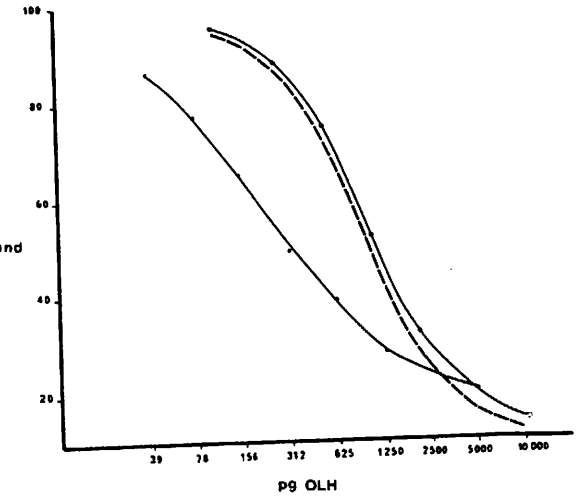

FIG. 2. Displacement curves using ovine LH (NIH-LH-S18) as standard and radioiodinated rat and ovine LH. (○ – – – ○): In this system antiovine LH antiserum (GDN 15) diluted 1:80,000 was incubated with ovine LH standard for 24 h at 25 C, followed by addition of rat [^{125}I]iodo-LH and incubation for 6 h at 25 C. The effect of 100 μl human plasma is shown by the broken line. (● —— ●): In this system conditions were identical to the above except for increasing the antiserum dilution to 1:80,000, utilizing ovine [^{125}I]iodo-LH, and reducing the total incubation volume to 450 μl.

to include plasma lacking immunological LH in standard tubes.

When ovine [^{125}I]iodo-LH was used instead of rat [^{125}I]iodo-LH the sensitivity of the assay was enhanced considerably (Fig. 2), since the antibody could be diluted to 1:80,000. In a comparison in 28 assays, 50% of ovine [^{125}I]iodo-LH was displaced by 601 ± 64 pg standard LH (mean ± SE) whereas 50% of rat [^{125}I]iodo-LH was displaced by 1101 ± 62 pg standard LH. The multiple buffer system employed by Niswender *et al.* (6) was also replaced in these assays by a more convenient single diluent buffer consisting of 0.04M sodium phosphate (pH 7.4), 0.15M NaCl, 0.1% NaN_3, 0.01M EDTA and 0.5% BSA which was utilized as diluent throughout. This assay system was used in all studies of LH in plasma samples from the different mammalian species (Fig. 5).

The ability of the assay to measure LH in plasma samples with good accuracy and reproducibility was tested in several ways. When plasma samples were assayed at different dilutions, the curves were parallel to the standard curves (see Fig. 5) and the values obtained were similar (see Table 1). Exogenous LH was also recovered quantitatively from plasma. When different amounts of ovine LH (x) were added to hyrax plasma and compared with the quantity assayed after subtraction of endogenous LH (y) a regression line with the equation $y = 0.99x + 0.04$ was obtained (Fig. 3). The correlation coefficient (r) was 0.99. The coefficient of variation within a single assay was 2.1%

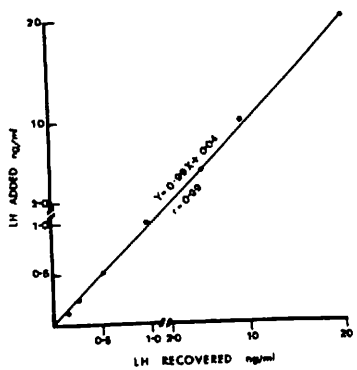

FIG. 3. Recovery of endogenous ovine LH added to hyrax plasma. LH recovered was calculated by subtraction of endogenous LH value from the total assayed value.

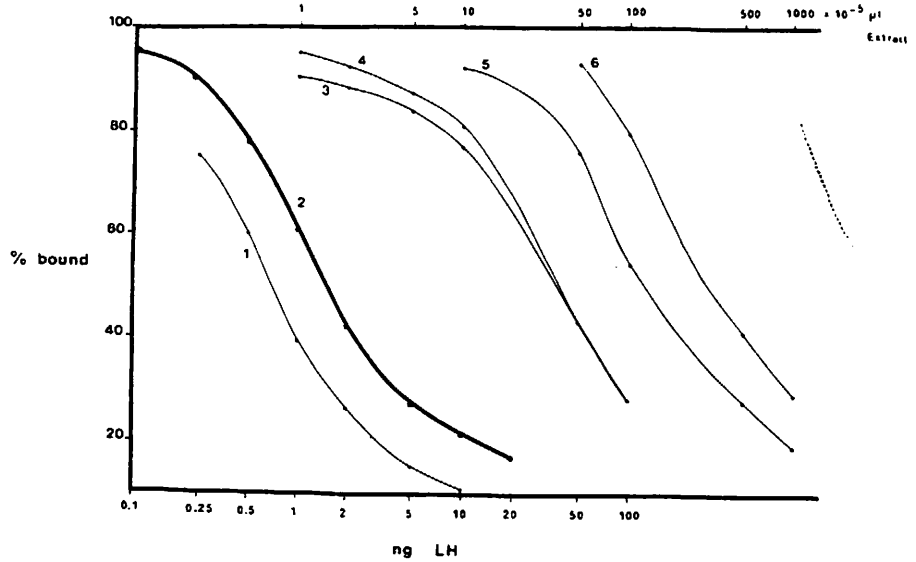

FIG. 4. Dose-response curves obtained with pituitary homogenates and purified hormones from various animals. Each point represents the mean of two determinations. 1, bovine LH (Carr CC-3); 2, ovine-LH (NIH-LH-S[illegible]); 3, rat-LH (NIAMD-rat-LH-3RP-1); 4, ovine pituitary homogenate; 5, thar pituitary homogenate; 6, hyrax pituitary homogenate. Ground-squirrel and hamster pituitary homogenates also yielded parallel curves (separate assay). Ovine FSH produced a parallel curve but the displacement was only 0.8% of that of the LH standard when comparing the relative masses required to inhibit binding of [^{125}I]iodo-LH by 50%. Rat [^{125}I]iodo-LH was employed in this study and the anti-LH antiserum diluted 1:20,000.

and the coefficient of variation when the same sample was assayed in five different assays was 18%.

Specificity and cross-reactivity

Displacement curves for LH in plasma and pituitaries of various species are shown in Figs. 4 and 5. Hyrax, thar, ground-squirrel, rat, hamster, ovine and bovine pituitary extracts gave displacement curves parallel to that of the ovine standards (Fig. 4). By contrast, up to 500 ng of hMG, hCG, hGH, hTSH, rGH and avian LH caused no significant displacement of the rat [^{125}I]iodo-LH. Ovine FSH (NIH-FSH-S10) produced a parallel displacement curve but 152 ng was required to displace 50% of [^{125}I]iodo-LH. Plasma from hyrax, sable, reedbuck, impala, tsessebe, spring-hare, ground-squirrel, thar, cheetah, the domestic horse and the laboratory rat and hamster all produced parallel displacement curves (Fig. 5, Table 1). The displacement curve of bontebok plasma was not parallel to that of the ovine standard (Fig. 5, Table 1).

Insufficient displacement of [^{125}I]iodo-LH occurred with all plasma samples collected from blesbok, roan antelope, bush baby, vervet monkey, rhino, porcupine and a hyena to allow assessment of parallelism. In the springbok 10, 25 and 50 μl of plasma induced increasing displacement of [^{125}I]-iodo-LH but, paradoxically, 100 μl and 150 μl of plasma actually enhanced binding of [^{125}I]iodo-LH.

In hyrax material the presence of relatively larger quantities of other protein hormones did not interfere in the quantitation of LH. Dilution of extracts of pituitaries from sexually quiescent hyrax containing relatively little LH still produced parallel displacement curves (Table 1). Similarly, quantitation of LH was not impaired when the TSH plasma concentration in hyrax was presumably stimulated by iv administration of 100 μg TRH (Fig. 5, Table 1). Plasma LH levels measured by radioimmunoassay corresponded with physiological state. Mean plasma LH was 2.4 ng/ml in sexually quiescent male hyrax while mean plasma LH was 12.1 ng/ml in sexually active males (Table 2). Plasma LH increased from basal levels of 0.9 ng/ml to 23.2 ng/ml after iv injection of 10 μg LHRH. Conversely, administration of 250 μg testosterone reduced mean plasma LH from 1.7 ng/ml to 0.7 ng/ml 6 h after im injection.

Biological activity of LH in pituitaries taken from sexually active and sexually quiescent male hyrax further validated the radioimmunoassay of LH since values obtained by the two methods were similar (Table 3). However, a discrepancy has been noted on this and a previous occasion in that at the height of the sexual season bioassay estimates of pituitary LH are lowest while radioimmunoassay results are highest (2). This discrepancy is the subject of a future publication.

A good correlation between physiological state and assayed plasma LH was apparent in the bontebok, spring-hare, thar, cheetah, domestic mare and laboratory rat. A signifi-

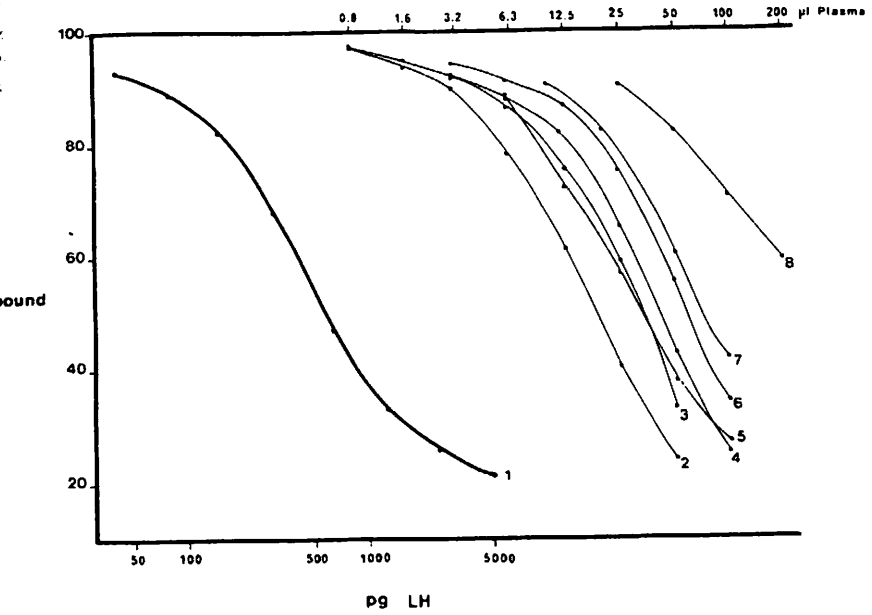

FIG. 5. Dose-response curves obtained with ovine LH (NIH-LH-S18) and plasma from various mammals. Each point represents the mean of two determinations. 1, ovine-LH (NIH-LH-S18) standard; 2, hyrax plasma after 10 μg LHRH and 100 μg TRH; 3, mountain reedbuck; 4, hyrax plasma; 5, ground-squirrel; 6, thar; 7, domestic mare; 8, bontebok. Plasma from spring-hare, sable, impala, tsessebe, cheetah and hamster also gave parallel curves, but are not shown here. A sample of human plasma induced no displacement in this assay. Ovine [^{125}I]iodo-LH was employed in this study and the antiserum diluted 1:80,000.

TABLE 1. Effect of dilution on the assay of LH in plasma and pituitary homogenates

Sample	μl/tube	Estimated LH ng/ml
Male hyrax plasma	10	22*
	20	20
	50	19
	200	20
	400	45
Male hyrax plasma after TRH and LHRH	6.3	32
	12.5	31
	25.0	34
Sexually quiescent male hyrax pituitary	0.0005	380
	0.001	440
	0.005	420
	0.01	450
Spring-hare plasma	0.8	368
	1.6	448
	3.2	400
	6.3	344
	12.5	336
Reedbuck plasma	6.3	22
	12.5	18
	25	18
	50	25
Thar	50	5.0
	100	4.6
	150	4.5
Bontebok plasma	25	2.8
	50	3.2
	100	2.8
	200	2.1
Ground-squirrel plasma	12.5	21
	25	20
	50	20
	100	22
Sable plasma	50	3.0
	100	3.3
Tsessebe plasma	50	1.5
	100	1.3

* Mean value for duplicate estimates.

In the majority of species, plasma with the highest level of LH was selected from a large number of animals for these studies.

cant rise in LH was observed after iv administration of 100 μg LHRH to male impala (Table 2).

Discussion

The radioimmunoassay described compares favorably with other radioimmunoassays for LH in precision, accuracy, specificity and sensitivity (3–10). The assay system finally adopted actually exhibits an increased sensitivity when compared with these radioimmunoassays and allows the detection of as little as 20 pg ovine LH standard as reported by Ciemanec and Niswender (15). The enhanced sensitivity is a result of iodinating highly purified ovine LH (LER-1056-C2) to a specific activity in excess of 300 μCi/μg, the use of greater dilutions of antiserum (to yield less than 30% binding of [^{125}I]iodo-LH in the absence of unlabelled LH) and a reduction in volume of reactants.

TABLE 2. LH in relation to physiological state in mammalian species

Species and reproductive state	No. samples	Plasma LH (ng/ml) Mean ± SE	Pituitary LH (μg/gland) Mean ± SE	Testosterone concentration (ng/ml) Mean ± SE
Male hyrax				
Sexually active	7	12.1 ± 4.7	194 ± 42	12 ± 1.8
Sexually quiescent	11	2.4 ± 0.9	76 ± 18	0.4 ± 0.4
Before LHRH	5	0.9 ± 0.2		
30 min after 10 μg LHRH	5	23.2 ± 3.2		
Before testosterone	6	1.7 ± 0.4		
6 h after 250 μg testosterone	6	0.7 ± 0.3		
Male bontebok				
Sexually active	6	2.2 ± 0.3		1.3 ± 0.3
Sexually quiescent	5	1.0 ± 0.3		0.3 ± 0.1
Male impala				
Before LHRH	20	1.0 ± 0.5		
90–120 min after 100 μg LHRH	12	4.5 ± 1.1		
Male spring-hare				
Sexually active	13	3.1 ± 5.0		2.5 ± 0.4
Sexually quiescent	22	1.4 ± 1.0		1.1 ± 0.5
Immature	6	0.6 ± 0.7		1.2 ± 0.7
Female spring-hare				
Pregnant	20	7.9 ± 2.5		
Lactating	6	1.2 ± 0.2		
Immature	9	1.2 ± 0.1		
Male thar				
Sexually active	7	12.6 ± 3.7	237 ± 47	10.0 ± 1.3
Sexually quiescent	5	0.9 ± 0.3	<8	1.2 ± 0.3
Immature	5	1.0 ± 0.3	56 ± 21	0.7 ± 0.2
Male cheetah				
Sexually active	6	2.2 ± 0.3		1.5 ± 0.3
Sexually quiescent	5	1.0 ± 0.3		0.3 ± 0.1
Mare estrus LH peak				
Day 0		16.0		
Day 2		45.0		
Day 4		44.0		
Day 6		22.0		
Day 8		7.8		
Male laboratory rat				
Normal	4	2.0 ± 0.5		
Estradiol implants	4	<0.5		
Castrated	6	7.5 ± 1.1		

The inability of hMG, hCG, hGH, rGH, TSH and avian LH to induce a significant displacement of [^{125}I]iodo-LH from antibody confirms the established specificity of GDN-15 antiserum (16). Moreover, the LH potency of NIH-FSH-S10 in the present radioimmunoassay corresponds with the OAAD estimated LH contamination of this preparation. These results, together with the observed changes in plasma LH in normal, castrated and estradiol-treated male rats (Table 2), confirm the comprehensive validation of the use of this antiserum for quantitating rat LH reported by Niswender *et al.* (6).

Increasing quantities of pituitary extracts and/or plasma from 14 of the 24 mammalian species studied, yielded displacement curves parallel to that of the ovine LH standard curve, suggesting that the hormone might be specifically quantitated in these species. In certain species (bontebok, impala, spring-hare, thar, cheetah, rat and horse) immunoassayable LH correlated well with reproductive state or treatment with LHRH or testosterone or estradiol (Table 2), suggesting that radioimmunoassay quantitation of LH is probably meaningful in these species.

Nevertheless, it should be strongly stressed that these criteria alone are insufficient to validate the radioimmunoassay of LH in these species. In the hyrax a more detailed examination of the specificity of the assay was undertaken. Dilution of pituitary extracts from sexually quiescent hyrax, in which the concentration of other protein hormones is relatively higher than in sexually active hyrax, still yielded parallel displacement curves (Table 1). Similarly, quantitation of LH was not impaired when plasma TSH was stimulated by iv administration of TRH (Fig. 5, Table 1). A good correlation between reproductive physiological state in the hyrax and immunoassayable plasma LH was also observed in that plasma LH was low in immature, sexually quiescent and testosterone-treated hyrax, while LH concentration was high in sexually active hyrax and markedly elevated after administration of LHRH (Table 2). Finally, pituitary LH estimates by radioimmunoassay were in good agreement with biological assay in spite of the fact that levels of LH, FSH and presumably other protein hormones varied in samples (Table 3). All of these data confirm the validity of the radioimmunoassay for quantitating LH in the hyrax. The fact that the anti-ovine LH serum appears to bind LH of the hyrax whose phylogenetic relationship with the Ovidae is remote adds further credence to the suggestion that this antiserum will quantitate LH in a variety of mammalian species.

TABLE 3. Comparison of radioimmunoassay and biological assay of hyrax pituitary LH content

Time when pituitaries collected*	LH by OAAD bioassay μg/mg	LH by RIA μg/mg	RIA LH / OAAD LH	FSH by bioassay μg/mg
Start of mating season	4.27	4.01	0.94	20.1
Sexually quiescent	1.77	1.84	1.04	20

* 4 pooled pituitaries in each group.

LH and FSH estimates expressed in terms of NIH-LH-S18 and NIH-FSH-S11.

Niswender *et al.* (15) and Midgley *et al.* (17) have reported that the antiserum used in the present study is specific for quantitation of LH of several mammalian species. The ability of the antiserum to bind LH from different species suggests that the amino acid sequence of the immunogenic region of LH to which the predominant antibodies in this antiserum were formed has been highly conserved during the course of mammalian evolution. The amino acid sequence is invariably conserved in those regions of proteins important for maintaining the correct conformation and charge of functional regions of the molecule (18). Thus, the GDN-15 anti-ovine LH serum most probably binds a region which plays a part in the maintenance of the correct conformation and/or charge essential for conveying biological activity.

In conclusion, the radioimmunoassay described can be used to quantitate specifically LH in the hyrax, and most likely in many of the other mammalian species studied. The potential therefore exists for fundamental

endocrine studies of the regulation of LH secretion in wild mammals.

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Biphasic Thyrotropin Suppression in Euthyroid and Hypothyroid Rats[1]

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ABSTRACT. To determine whether thyrotropin (TSH) suppression was characterized by a rapid and slow component in euthyroid rats, TSH concentrations were measured in concentrates of plasma pooled from groups of 8 euthyroid rats 24 h after injection of a single dose of triiodothyronine (T_3). Plasma TSH decreased after T_3 injection to values 3.2–5.9% of pre-T_3-injection concentrations. The presence of TSH in plasma after single T_3 injection and its absence (<0.25% of euthyroid values) after euthyroid rats were treated with T_3, 2–4 μg/day for 43 days, indicate that, as in the hypothyroid rat, TSH suppression has both a rapid and slow component in the euthyroid rat. A small but statistically insignificant increase in TSH secretion occurred after injection of a high dose of thyrotropin-releasing hormone (TRH; 1 μg/100 g BW) into T_3-treated euthyroid or hypothyroid rats. This suggested that endogenous TRH did not play an important role in maintaining TSH secretion in the presence of high plasma T_3 concentrations.

The metabolic clearance rate (MCR) of TSH was measured to determine whether altered rates of TSH metabolism could account for the 30–100-fold greater TSH concentration observed after 24 h in T_3-injected hypothyroid rats as compared to T_3-injected euthyroid rats. Neither a mean 40% decrease in the MCR of TSH nor a 4-fold increase in number of thyrotrophs in hypothyroid rats could account for the large difference in residual TSH concentration. The TSH secretion rate per thyrotroph in non-injected or T_3-injected hypothyroid rats appears to be 4–8-fold greater than in euthyroid rats. (*Endocrinology* 101: 769, 1977)

WE HAVE recently demonstrated both a rapid and a slow component of thyrotropin (TSH) suppression by 3,5,3′-triiodothyronine (T_3) in hypothyroid rats (1). The major decrease in plasma TSH concentration occurred rapidly (hours) after T_3 injection. After T_3-induced rapid suppression, however, plasma TSH concentrations remained readily measurable at values approximately 10% of initial concentrations. A slow component of TSH suppression was demonstrated in these animals by the long-term (15–25 days) daily T_3 administration that was required to decrease the plasma residual TSH to undetectable values. Only a rapid component of TSH suppression was noted in euthyroid rats. Since TSH concentrations decreased to the undetectable assay range after T_3 injection, we could not establish whether biphasic TSH suppression occurred in these animals or was characteristic of the hypothyroid state only. Indeed, published reports suggest that some TSH may be secreted in human subjects despite elevated concentrations of thyroid hormones (2–6). The present report describes measurements of the TSH concentration in concentrates of plasma from T_3-injected euthyroid rats. Continued TSH secretion after rapid TSH suppression but not after prolonged T_3 treatment was demonstrated in these animals. The pituitary response to administered thyrotropin-releasing hormone (TRH) in TSH-suppressed animals was also studied. Finally, measurements of the metabolic clearance rate of TSH indicate that the greater plasma TSH concentration observed in hypothyroid rats than in euthyroid rats after rapid suppression was not adequately explained by a slower rate of TSH metabolism. The data suggest that the greater TSH concentration after rapid suppression in hypothyroid rats is due mainly to increased pituitary secretion.

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