Characterization of a radioimmunoassay for ovine FSH utilizing an anti-bovine FSH serum

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Summary. The sensitivity of the assay, utilizing a bovine FSH antiserum and ^{125}I -labelled bovine FSH tracer, is 5 ng NIH-FSH-S6/ml, and cross-reactivities of other ovine pituitary glycoprotein and protein hormones (LH, TSH, growth hormone and prolactin) are less than 1.0%. Both pituitary and serum FSH of the sheep cross-react in a manner parallel with that of standard NIH-FSH-S6. Serum FSH concentrations in 4 ewes at various times after the onset of oestrus peaked more or less coincidentally with the preovulatory increase of serum LH. The serum levels of FSH and LH did not correlate proportionally but fluctuated considerably in individual animals. The radioimmunoassay system appears to be sensitive and specific for measuring serum concentrations of FSH in rams and ewes in different physiological states.

Introduction

The development of an homologous radioimmunoassay for ovine FSH has met with difficulties because of the extensive cross-reactivity of ovine FSH with the other ovine glycoprotein hormones, LH and TSH (L'Hermite, Niswender, Reichert & Midgley, 1972; Cunningham & Hebert, 1973). For this reason, many heterologous radioimmunoassays using antisera against FSH of one species in combination with labelled FSH tracer of a different species have been developed to measure serum levels of FSH in the sheep (L'Hermite *et al.*, 1972; Salamonsen *et al.*, 1973; McNeilly, McNeilly, Walton & Cunningham, 1976). Cheng (1978a) developed an homologous radioimmunoassay for bovine FSH and observed that ovine FSH cross-reacted significantly in this system. The present paper describes the characterization of this radioimmunoassay for measuring ovine FSH, utilizing bovine FSH antiserum and ^{125}I -labelled bovine FSH as a tracer, and its application to monitor serum levels of FSH in ewes at oestrus.

Materials and Methods

Materials. Highly purified bovine FSH (potency = $160 \times$ NIH-FSH-S1) and its rabbit antiserum were obtained as described previously (Cheng, 1976, 1978a). Purified ovine FSH (LER-1491) and reference standards of ovine FSH (NIH-FSH-S6), LH (NIH-LH-S14; LH-S18; LH-S19), TSH (NIH-TSH-S8), growth hormone (NIH-GH-S11) and prolactin (NIH-PRL-S12) were obtained from the National Institute of Arthritis, Metabolic and Digestive Diseases, National Institutes of Health, Bethesda, Maryland. Bovine serum albumin (fraction V) was purchased from Miles Laboratory, Kankakee, Illinois. Na^{125}I (carrier free) was purchased from New England Nuclear, Boston, Massachusetts. Sephadex G-100 was from Pharmacia (Canada) Ltd, Dorval, Quebec. All other reagents and chemicals were reagent grade.

Radioimmunoassay for ovine FSH. The radioimmunoassay system was almost identical to the homologous radioimmunoassay for bovine FSH (Cheng, 1978a), except that crude ovine FSH (NIH-FSH-S6) was used as standard instead of bovine FSH (NIH-FSH-B1). A specific antiserum was obtained from a rabbit by immunizing with highly purified bovine FSH (Cheng, 1978a). To obtain optimal sensitivity, the antiserum was used at an initial dilution of 1:60 000. Highly purified bovine FSH was labelled with ^{125}I by the lactoperoxidase method as described previously (Cheng, 1975), and used as tracer. All dilutions of antiserum, hormone standards and serum samples were made with 0.02 M-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl, 1% BSA and 0.1% sodium azide. A double-antibody system was used. The displacement of antibody-bound ^{125}I -labelled bovine FSH by various concentrations of standard ovine FSH is expressed as a percentage of the ratio B/B_0 , where B is the amount of ^{125}I -labelled FSH bound to antibody in the presence of different concentrations of hormone standard and B_0 is the total amount bound in the absence of unlabelled hormone.

Radioreceptor assay for FSH. A radioreceptor assay specific for FSH (Cheng, 1975), utilizing partly purified plasma membranes from bovine testis and ^{125}I -labelled bovine FSH, was used to assess the specificity of the radioimmunoassay for ovine FSH by monitoring FSH activity in the eluants of lamb pituitary extracts after fractionation by gel filtration on Sephadex G-100. Ovine FSH (NIH-FSH-S6) was used as standard.

Radioimmunoassay for ovine LH. Serum LH concentrations were determined by a double-antibody radioimmunoassay for ovine LH as described by Niswender, Reichert, Midgely & Nalbandov (1969) and modified by Howland (1972). The anti-cvine LH serum (GDN No. 15) was used at an initial dilution of 1:100 000. LH measurements were expressed in terms of ng NIH-LH-S14/ml. The minimum detectable level, defined as 95% of initial binding (B/B_0), was 0.2 ng/ml. Intra- and inter-assay coefficients of variation based on 6 replicate determinations of a single serum pool with a mean concentration of 1.65 ng/ml were 9.7 and 7.6%, respectively.

Gel filtration on Sephadex G-100. Immediately frozen lamb pituitaries were extracted by homogenizing in 0.02 M-phosphate-buffered saline at a concentration of 100 mg tissue/ml buffer. The pituitary extract was then fractionated by gel filtration on Sephadex G-100 in 0.5% NH_4HCO_3 buffer, pH 8.2, at 4°C. FSH activity in the eluants after fractionation was monitored by radioimmunoassay and radioreceptor assay, using the same NIH-FSH-S6 standard. The appropriate fractions of FSH activity were pooled and freeze-dried for further characterization. Distribution of proteins in the eluants after column chromatography was monitored by absorbance at 278 nm in a Beckman Model 25 spectrophotometer.

Serum concentrations of FSH and LH in cyclic ewes. Blood samples were taken from 4 3-year-old Finnish Landrace ewes at 20-min intervals for 16 h on the day of oestrus, which was detected by using a raddled vasectomized ram. The blood samples were allowed to clot at 4°C for several hours and the serum was removed and stored at -20°C until assay.

Results

Sensitivity, specificity, accuracy and precision

Text-figure 1 shows the dose-response curves of the highly purified ovine FSH (NIH-LER-1491) and crude standard ovine FSH (NIH-FSH-S6). The limits of detection for purified and standard ovine FSH were 0.3 and 5.0 ng/ml, respectively, which could be significantly discriminated from zero at the 95% confidence level on the basis of twice the standard deviation of the zero value.

Ovine pituitary growth hormone (NIH-GH-S11), prolactin (NIH-prolactin-S12) and thyrotrophin (NIH-TSH-S8) at concentrations of 1000 ng/ml showed no significant displacement. Only very slight cross-reactivity was observed for ovine LH (NIH-LH-S18 and -S19). This radioimmunoassay therefore appears to be specific for ovine FSH.

Text-fig. 1. Dose-response curves for ovine glycoprotein and protein hormones in the radioimmunoassay, utilizing a bovine FSH antiserum and ^{125}I -labelled bovine FSH tracer. Details of the sources of various hormones are given in 'Materials and Methods'. B = total amount of ^{125}I -labelled bovine FSH bound to antibody; B_0 = amount of ^{125}I -labelled bovine FSH bound to antibody in the absence of unlabelled hormone.

Table 1 depicts the recovery of added ovine FSH to serum samples; the recoveries were within the precision of this assay system. Little or no interference of the serum component was observed.

Table 1. Recovery of various amounts of ovine FSH (FSH-S-6) added to serum samples of 3 different ewes (A, B and C) with different initial concentrations of FSH

FSH added (ng/ml)	Total FSH detected (ng/ml)		
	A	B	C
0	17.0	66.2	190.4
10	25.5	75.6	198.8
25	40.8	90.5	213.0
100	118.8	170.3	300.0

Values are means of duplicate determinations.

Estimation of the intra- and inter-assay precision was carried out by measuring the variation within and between assays of samples of 10 and 100 ng/ml of the ovine FSH standard in assay buffer; the values obtained were $\pm 7\%$ ($n = 15$) and $\pm 11\%$ ($n = 16$), respectively.

FSH activity in pituitary extracts by radioimmunoassay and radioreceptor assay

Immediately frozen anterior pituitary lobes of lambs were thawed, rinsed with 0.15 M-NaCl and homogenized individually in 0.02 M-phosphate-buffered saline, pH 7.0, at a concentration of 100 mg wet weight/ml buffer. After centrifugation at 10 000 g, the supernatant was collected and fractionated by gel filtration on a column of Sephadex G-100. The distribution profiles of proteins and FSH activities in the eluates showed only one peak of coincident FSH activity in each of the assay systems (Text-fig. 2). The values obtained by radioimmunoassay, however, were approximately 10–15% higher than those obtained by radioreceptor assay. Nevertheless, this radioimmunoassay appears to measure specifically FSH activity in pituitary extracts. The FSH fractions (136–168) were pooled and freeze-dried for further characterization.

Text-fig. 2. Elution profile of FSH activities (----), as measured by radioimmunoassay (RIA) and radioreceptor assay (RRA), in eluates of lamb pituitary extracts after gel filtration on Sephadex G-100. Distribution of proteins (—) was monitored by absorbance at 278 nm.

Immunological characterization of serum and pituitary FSH

The specificity of the radioimmunoassay was further characterized by measuring FSH activities in sera from ewes and rams. All the serial dilution and the freeze-dried FSH fractions of lamb pituitaries (Text-fig. 2) were parallel to that of the ovine FSH standard (NIH-FSH-S6), thus indicating identical immunological cross-reactivities between serum FSH, pituitary FSH and the FSH standard in this radioimmunoassay system (Text-fig. 3).

Text-fig. 3. Comparison of the inhibition curve at different dilutions for standard NIH-FSH-S6, FSH in 3 samples of serum from 3 sheep and FSH in 2 lamb pituitary extracts. B = total amount of ^{125}I -labelled bovine FSH bound to antibody; B_0 = amount of ^{125}I -labelled bovine FSH bound to antibody in the absence of unlabelled hormone.

Serum levels of FSH and LH at oestrus

Basal and peak levels of FSH varied considerably between individual animals, ranging from 10–50 ng/ml to 60–200 ng/ml, respectively (Text-fig. 4). A definite peak of serum FSH at oestrus was observed in all 4 animals and was more or less coincident with the preovulatory surge of LH. However, serum levels of FSH and LH did not parallel each other exactly in the 4 animals. This observation also supports the specificity of the radioimmunoassay in measuring serum FSH concentrations without significant interference from LH in physiological studies of sheep.

Text-fig. 4. Profiles of serum levels of FSH (○) and LH (●) over a 16-h period in 4 cyclic ewes at oestrus. Serum samples were collected at 20-min intervals from the onset of oestrus.

Discussion

Despite the availability of highly purified ovine FSH (Jiang & Reichert, 1964; Papkoff, Gospodarowicz & Li, 1967; Sherwood, Grimek & McShan, 1970), very few specific homologous radioimmunoassays using ovine FSH and unabsorbed antisera have been described

(Crim & Geschwind, 1972; Kragt & Cons, 1973). The main problem is the cross-reactions between FSH, LH and TSH (Bailly du Bois, Kerdelhue & Jutisz, 1970; L'Hermite *et al.*, 1972; Cunningham & Hebert, 1973). To circumvent the problem of specificity, heterologous radioimmunoassays using either ovine FSH antisera and ^{125}I -labelled human or rat FSH tracer (L'Hermite *et al.*, 1972; Kerdelhue, Kann & Jutisz, 1972; Hopkinson & Pant, 1973; Salamonsen *et al.*, 1973) or human FSH antisera and ^{125}I -labelled ovine or rat FSH tracer (Salamonsen *et al.*, 1973; McNeilly *et al.*, 1976) and rat FSH antisera with ^{125}I -ovine FSH (Dobson & Ward, 1977) have been developed. Some of these heterologous systems appear to be specific for measuring serum levels of FSH in the sheep.

The present system of radioimmunoassay, utilizing a bovine FSH antiserum and an ^{125}I -labelled bovine FSH tracer, has been characterized as being highly specific for ovine FSH. Cross-reaction with other ovine glycoprotein and protein hormones is negligible (Text-fig. 1). The specificity of this antiserum for bovine FSH has been fully characterized (Cheng, 1978a), and the chemical nature of bovine and ovine FSH has been shown to be very similar (Cheng, 1976, 1978b). Similarities between these hormone molecules probably account for the high cross-reactivity of ovine FSH with the bovine FSH antiserum. The discrepancy (10–15%) between values for FSH on crude pituitary extracts obtained by radioimmunoassay and radioreceptor assay (Text-fig. 2) does not seem to be due to interferences from other pituitary glycoprotein hormones since the elution profiles of FSH activity monitored by both assays were symmetrical peaks and coincident with each other. The elution volumes of bovine FSH, LH and TSH varied slightly upon gel filtration on Sephadex G-100 (Cheng, 1976). It has generally been established that immunoassays and receptor assays measure different aspects of the configuration of the protein and this may account for the differences of measurements by these systems. Preparations of ovine LH (NIH-LH-S18 and NIH-LH-S19) and highly purified bovine LH (Cheng, 1978a) showed a cross-reactivity of less than 1.0% (Text-fig. 1), but NIH-LH-S14 has a 25–30% level of cross-reactivity in this assay (unpublished observation). As little cross-reaction was observed in three preparations of purified ovine and bovine LH, this high level of cross-reaction of NIH-LH-S14 is probably due to contamination of some immunoreactive components, possibly FSH, either in the preparation or during subsequent handling of this material in the laboratory.

The sensitivity of this radioimmunoassay (5.0 ng NIH-FSH-S6/ml) appears to be considerably better than that of most of the other heterologous systems previously reported (20 ng NIH-FSH-S4/ml: L'Hermite *et al.*, 1972; 13 ng NIH-FSH-S6/ml: Salamonsen *et al.*, 1973; 15 ng NIH-FSH-S8/ml: McNeilly *et al.*, 1976). This radioimmunoassay has also been demonstrated to be precise and accurate. The recovery of exogenous standard FSH added to serum samples was satisfactory over the working range of the assay curve, and the dilution curves of serum and partly purified pituitary extracts were parallel to that of the standard ovine FSH. The results for serum FSH measurements in cyclic ewes are in good agreement with those reported previously by other investigators (L'Hermite *et al.*, 1972; Hopkinson & Pant, 1973; Salamonsen *et al.*, 1973; McNeilly *et al.*, 1976; Dobson & Ward, 1977). However, the blood levels of FSH varied greatly in the 4 ewes and serum FSH did not change identically or proportionally to the levels of LH, providing further support for the specificity of the radioimmunoassay for measuring serum FSH. These observations indicate the lack of interference in this radioimmunoassay by different serum components. This radioimmunoassay therefore appears to be suitable for measuring serum concentrations of FSH in rams and ewes.

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