The measurement of ovine follicle stimulating hormone (FSH) bioactivity utilizing a recombinant human FSH receptor bioassay

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ABSTRACT

Recently a bioassay for human FSH has been described based on FSH stimulation of cyclic AMP production by a Chinese hamster ovary (CHO/FSH-R) cell line stably expressing the human FSH receptor (FSH-R cells). In this study, the viability of this bioassay for measuring ovine FSH was examined. Freshly thawed FSH-R cells were bulked up in culture, and aliquots of 250,000 cells/well dispensed into 48 well culture dishes and incubated overnight at 37°C. The assay culture media was then replaced with 0.25 ml fresh media (α-MEM + 0.1% BSA + 0.25 mM 3-isobutyl-1-methyl-xanthine) containing varying doses of FSH preparations, and the cells incubated for 4 h at 37°C. The assay culture media was then removed and stored frozen at -20°C until assayed for cyclic AMP by radioimmunoassay. A standard ovine FSH preparation NIH-FSH-RP2 and Ovagen, a cruder commercial preparation used for superovulation, stimulated cAMP by FSH-R cells in a dose dependent manner. Other ovine pituitary hormones including LH, GH, TSH, prolactin, α-FSH and β-FSH had no stimulatory effect on cAMP production. The lowest dose of ovine FSH that could be measured with the FSH-R cells was 0.8 ng/ml of RP2 with an intra-assay CV of 8.5%. Serum samples taken from ovariectomised ewes and fractions from a pituitary extract subjected to isoform separation by ion-exchange chromatography were assayed using the FSH-R cells. The results showed good correlation with FSH radioimmunoassay data from the same samples ($r=0.96$, $P<0.01$), with the levels of circulating FSH dropping over time. It is concluded that this convenient and robust bioassay will have considerable application in investigations of ovine FSH bioactivity.

Keywords: follicle stimulating hormone; bioassay; cyclic AMP

INTRODUCTION

Follicle stimulating hormone (FSH) is involved in the regulation and maintenance of essential reproductive processes, including gametogenesis, follicular development and ovulation. FSH is a glycoprotein hormone produced in the anterior pituitary, and exists as a family of isohormones, which differ in their oligosaccharide structures. These FSH isoforms exhibit differences in receptor-binding activity, plasma half-life, and *in vivo* and *in vitro* bioactivity.

While the heterogeneity of FSH is well established, the physiological significance of the isoforms is unclear. FSH is traditionally measured by radioimmunoassay (RIA). However, there is increasing evidence that RIA measurements do not always reflect accurately the bioactivity of the different isoforms of FSH. Thus there is considerable interest in developing bioassays to measure FSH. Unfortunately, most bioassays for FSH lack the sensitivity for measuring the relatively low levels of FSH found in serum. Recently, however, a novel bioassay for measuring human FSH has been developed utilising a Chinese hamster ovary (CHO/FSH-R) cell line which stably expresses the human FSH receptor coupled to adenylate cyclase (Albanese et al., 1994). In this study we have investigated the viability of this assay for measuring ovine FSH, and in particular for measuring FSH in serum.

MATERIALS AND METHODS

The CHO/FSH-R cells (American Type Culture Collection, Rockwell, USA) used in the bioassay were grown in αMEM (Gibco/BRL Grand Island, USA) supplemented with 10% fetal calf serum (Gibco/BRL Grand Island, USA), penicillin, streptomycin, and geneticin, subsequently referred to as culture medium. Purified ovine FSH (NIH-FSH-RP2) used to standardise the bioassay, ovine GH (NIH-GH-S11) and ovine prolactin (NIH-P-S13) was obtained from the National Hormone and Pituitary Program of the NIH (Bethesda, MA, USA). Ovagen, a crude pituitary FSH preparation, was obtained from Immuno Chemical Products Ltd. (ICP, Auckland, NZ). Ovine TSH (NIADDK-oTSH-12) and ovine LH (NIADDK-oLH-25) was obtained from NIADDK (Torrance, CA, USA). Vasopressin was purchased from Sigma (St. Louis, MO, USA). α-FSH and β-FSH were both obtained from Bioscan (Quebec, Canada). Ovine serum samples were obtained from ovariectomised ewes which were subsequently hypophysectomised. Fractions from an ovine pituitary extract subjected to isoform separation on an ion exchange column was also tested.

The CHO/FSH-R cell bioassay was performed as described by Albanese et al., (1994). The assay is based on exogenous FSH interacting with the recombinant FSH receptor expressed on the CHO cell surface and stimulating cAMP production. Briefly, 2.5×10^5 cells were dispensed into 48 well culture plates. The plates were then incubated

for 16-20 hr at 37°C in a 5% CO_2 atmosphere. The medium was then replaced with 250 µl of αMEM containing 0.25 mM 3-isobutyl-1-methyl-xanthine (IBMX) obtained from Sigma (St. Louis, MO, USA), 0.1% bovine serum albumin (BSA) obtained from ICP (Auckland, NZ) and penicillin, subsequently referred to as assay medium. NIH-FSH-RP2 standards (0, 0.021, 0.051, 0.128, 0.32, 0.8, 2 and 5 ng/well) prepared in 0.5 mM PBS containing 1% BSA or sample solutions were then added and incubated for a further 4 h. The assay medium was then removed and stored frozen until assayed for extra-cellular cAMP by RIA (McNatty et al.,1989).

RESULTS

The addition of FSH resulted in a dose-dependent increase in cAMP production in comparison to control wells in serum-free assay medium. Addition of serum reduced this FSH-dependent response and shifted the dose-response curve to the right, with the amount of repression proportional to the volume of serum added.

Figure 1 shows the dose-dependent increase in cAMP production elicited by increasing amounts of NIH-FSH-RP2 and Ovagen. The ovine FSH standard has a higher biopotency than that of Ovagen, requiring lower concentrations of FSH to elicit the same response in cAMP. The specificity of the recombinant FSH receptor cell line to FSH was examined by testing the response to preparations of ovine prolactin, LH, TSH, GH, vasopressin, and the isolated α and β sub-units of FSH. In each case, the dose response was minimal with a cross-reactivity of less than 0.1%.

The plasma levels of FSH following hypophysectomy of ovariectomised ewes are shown in Figure 2. FSH

FIGURE 1: Effect of increasing doses of FSH and other pituitary hormone preparations on cAMP production by CHO/FSH-R cells. Values represent means of 3 replicate wells. The coefficient of variation at each point was <12%.

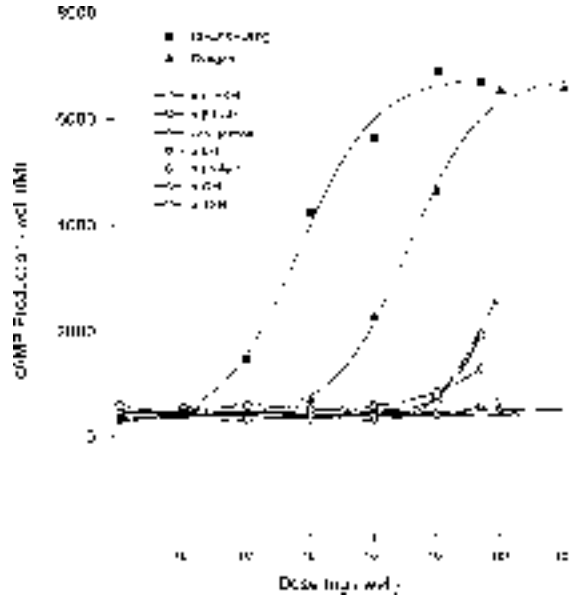


FIGURE 2: Serum FSH concentrations in ovariectomised ewes following hypophysectomy as measured by RIA or bioassay. FSH values are expressed as a percentage of the mean FSH concentration pre-surgery. Values are means of 5 ewes with vertical lines showing the standard error.

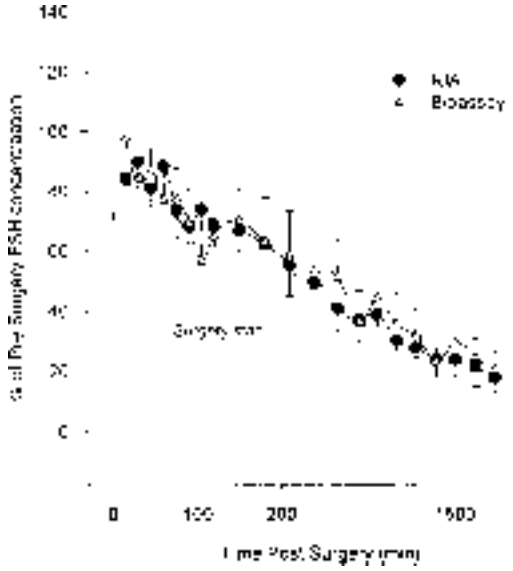
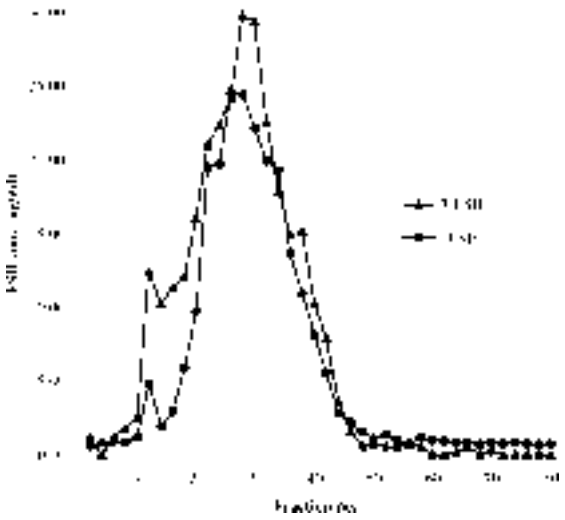


FIGURE 3: Bio- and Immuno- FSH (B-FSH and I-FSH respectively) levels in a pituitary extract subjected to isoform separation by ion-exchange chromatography. Values represent means of 3 replicate wells. The coefficient of variation at each point was <10%.

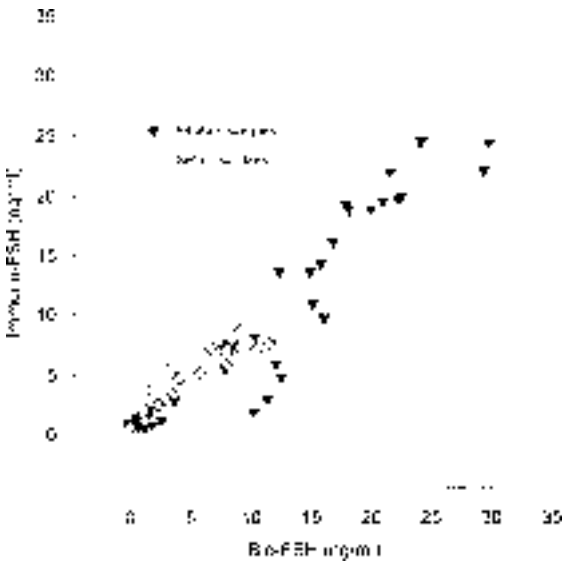


was measured by both RIA and bioassay. Both FSH assays demonstrate that following hypophysectomy there was a gradual decline in plasma FSH concentrations, as would be expected following removal of the source of FSH production. Moreover, the profile of the decline measured by both RIA and bioassay were similar.

Figure 3 shows the levels of FSH, measured by RIA and bioassay, in an ovine pituitary extract which had been passed through an ion exchange column to separate the various isoforms based on charge. The FSH profile of the fractions were similar whether the FSH was measured by bioassay or RIA.

The correlation between the levels of FSH in serum and pituitary samples measured by RIA and bioassay is

FIGURE 4: Correlation between FSH concentrations measured by RIA and bioassay. Values are means of 3 replicate wells and show correlations between bio- and immuno-FSH concentrations in serum and pituitary samples (r=0.96, P<0.01). The diagonal line represents a correlation of 1:1 between bio- and immuno- FSH concentrations.



shown in Figure 4. The overall correlation between the 2 assays is high. However, where differences do occur, this may be indicative of the different aspects of FSH activity measured by RIA and bioassay.

DISCUSSION

The results of this study show that the CHO/FSH-R cell bioassay which was originally developed for measuring human FSH is also suitable for measuring ovine FSH. Important features of this cell line include long-term culture stability in terms of both cell viability and FSH receptor expression, and elimination of primary tissue culture.

The CHO/FSH-R cell line has been validated for use in a bioassay based upon a number of important properties. Firstly, the cell line has been shown to be stable in culture and does not exhibit any changes in hormone responsiveness even after 12 months of continuous passage. Secondly, the cell line responds to FSH in a dose dependent fashion, but does not respond to other glycoprotein hormones at physiological concentrations and only minimally at supraphysiological concentrations. Thirdly, the sensitivity of the bioassay is high enough to allow accurate measurements of FSH at levels which are relevant *in vivo* (1-10 ng/ml). Furthermore, the amount of bioactive FSH measured by the receptor cell line correlates well with that measured by FSH RIA. This is evident in the FSH profiles obtained by both bioassay and RIA for serum and pituitary samples which show good agreement. The screening of purified FSH isoforms using this assay has been confirmed, and in conjunction with molecular biology techniques, may allow correlations of hormone structure and function.

We have found the recombinant FSH receptor bioassay to be more sensitive and robust than the rat Sertoli cell aromatase bioassay for FSH currently used in our laboratory (unpublished data). The intra-assay coefficient of variation and sensitivity of the CHO/FSH-R cell assay are 8.5% and 0.8 ng/ml respectively, while those of the Sertoli cell assay are 18.7% and 2.3 ng/ml respectively. In addition, the working range of the CHO/FSH-R cell bioassay (1-800 ng/ml) is much greater than that of the Sertoli cell aromatase bioassay (2.3-100 ng/ml). Furthermore, the CHO/FSH-R cell bioassay eliminates the need for animals as a source of material for primary cell cultures associated with the Sertoli cell bioassay.

Therefore the CHO/FSH-R cell bioassay reported here should provide an effective tool for studies of FSH structure and function.

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