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Regulation of LH Beta Subunit mRNA in The Sheep Pituitary Gland During Different Feedback States of Estradiol

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SUMMARY The feedback effects of gonadal steroids on the amounts of in vitro translated luteinizing hormone (LH) beta subunit were examined using cell-free assays. These amounts were then correlated with serum and pituitary concentrations during various feedback states. RNA was prepared, translated and products identified by immunoprecipitation and gel electrophoresis. The amounts of beta subunit varied in a pattern similar to that observed for alpha subunit. In ovariectomized ewes, the amounts of beta were 2-3X those seen in negative feedback groups and slightly more than those seen in animals exhibiting an LH surge. The pituitary LH concentration in ovariectomized ewes was also higher than those seen in the other groups, however, the serum concentrations in the positive feedback group were the highest of all groups. These results provide evidence for: 1) a separate, but coordinate, control of gonadotropin subunit synthesis; and 2) a contribution of subunit synthesis to the effects of positive and negative steroid feedback on pituitary LH amounts.

The structural and biological properties of the pituitary glycoprotein hormones have been well characterized. This family of hormones consists of three pituitary glycoproteins, LH[1], FSH and TSH, as well as CG from the placenta. Each of these contain two subunits (α and β), that are nonidentical and noncovalently linked. Within a species, the α subunits are derived from a single gene (2,3), while the beta subunits possess unique structures and, confer the biological specificity of the individual hormones (1).

These hormones represent an intriguing model for the study of the regulation of gene expression, due to separate subunit genes, and

[1] LH, luteinizing hormone; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone; CG, chorionic gonadotropin; GnRH, gonadotropin releasing hormone; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; RCM, reduced, carboxymethylated, OVX, ovariectomized; PRL:prolactin; GH, growth hormone.

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numerous effectors that regulate the physiological roles of the hormones (4-8), by affecting the levels of mRNAs for the gonadotropin subunits (9-12).

Previous studies from this laboratory have indicated that steroids affect the amounts of alpha subunit mRNA (10,11). In this study, we have examined these effects on the level of beta subunit mRNA in these ovine pituitaries, and correlated these values with the amounts of pituitary and serum. The results indicate that the amounts of beta mRNA are regulated in a similar manner to alpha mRNA, supporting the hypothesis that the subunit mRNAs are separately, but coordinately, regulated.

MATERIALS AND METHODS

Animals - All animals were sexually mature Suffolk ewes. The experimental animal groups were those used previously (10,11), and included three stages of gonadal steroid feedback. The ovariectomized ewes (OVX) represented a no feedback condition. Another group of ovariectomized ewes were treated with estradiol for 15 days to create a negative feedback condition. In addition, a group of normal anestrous ewes are included as a negative feedback group. The positive feedback group consisted of anestrous ewes treated with estradiol to elicit an LH surge (11).

Pituitary RNA Isolation - Pituitaries were removed at the time of sacrifice, dissected free of membranes and fatty material, and extracted with a phenol mixture as described previously (14). Yields were routinely 1-2 mg/gm of pituitary tissue.

Radioimmunoassays - Blood samples (5 ml) were collected and assayed as described previously (15, 16). The sensitivities of the serum LH assays (0.25 ng of NIH-LH-S12 per ml) were similar to those reported earlier (10).

Product Identification and Quantitation - Products were identified using immunoprecipitation and SDS-PAGE (14). Specific antibody to reduced, carboxymethylated (RCM) oLH beta subunit (supplied by Dr. Darrell Ward, University of Texas) was generated and characterized in the laboratory of the author (TDL). A fluorograph containing immunoprecipitates of *in vitro* translated pituitary proteins is shown in Figure 1. The specificity of the beta immunoprecipitate is demonstrated by: 1) its absence when the specific antibody is replaced with normal rabbit serum; and 2) competition of the band by a 100-fold excess of unlabeled antigen. Quantitation was achieved by excision of the gel slice containing the radioactive product, treatment with 500 ul Protosol (New England Nuclear) at 50^{o} for 2 hr and counted in 10 ml of Econofluor (New England Nuclear). Gel slices of equal dimensions were used to standardize recoveries, and included background counts from gels not containing specific radioactive products.

Data Analysis - Extracted RNA from individual pituitaries was assayed in separate translation assays. These assays demonstrated a coefficient of

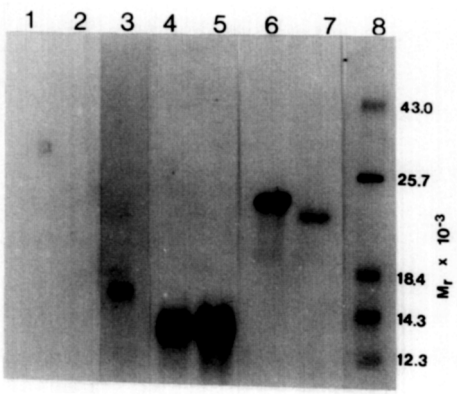

FIGURE 1. Fluorograph of SDS-PAGE analysis of immunoprecipitated cell-free products encoded by ovine pituitary RNA. Cell-free products were reacted with normal rabbit serum (lane 1), bovine RCM LH beta subunit antisera in the presence of 50ug of LH beta subunit (lane 2), bovine RCM LH beta subunit antisera (lane 3), bovine RCM LH alpha subunit antisera in the presence of 50ug of LH beta subunit (lane 4), bovine RCM LH alpha subunit antisera (lane 5), ovine prolactin antisera (lane 6) and ovine growth hormone antisera (lane 7). Lane 8 contains C-labeled protein standards (Bethesda Research Laboratories): ovalbumin (43000); alpha-chymotrypsinogen (25700); beta-lactoglobulin (18400); lysozyme (14300); and cytochrome C (12,300).

variation of 10-15%. Immunoprecipitated hormone amounts and RIA data are expressed as the mean ± SEM.

RESULTS

Pituitary and Serum LH Concentrations - Figure 2 depicts the relationship of pituitary LH concentrations and serum LH concentrations. In the ovariectomized ewes, LH amounts in serum and pituitary were 17.25 ± 4.29 ng/ml and 1279 ± 209 ug/g, respectively. Serum LH was decreased significantly, to less than 0.3 ng/ml, in both the estradiol-treated ovariectomized animals and the anestrous ewes while pituitary LH was also decreased in these two groups to less than 50% of the values observed in the ovariectomized group. Animals representing a positive feedback state exhibited much higher serum LH concentrations (49.49 ± 5.60 ng/ml) than the negative feedback ovariectomized groups. However, pituitary LH in this group (569 ± 162 ug/gm) was not significantly different from the two negative feedback groups (Fig. 2), and, in fact, was less than one-half that observed in the ovariectomized ewes.

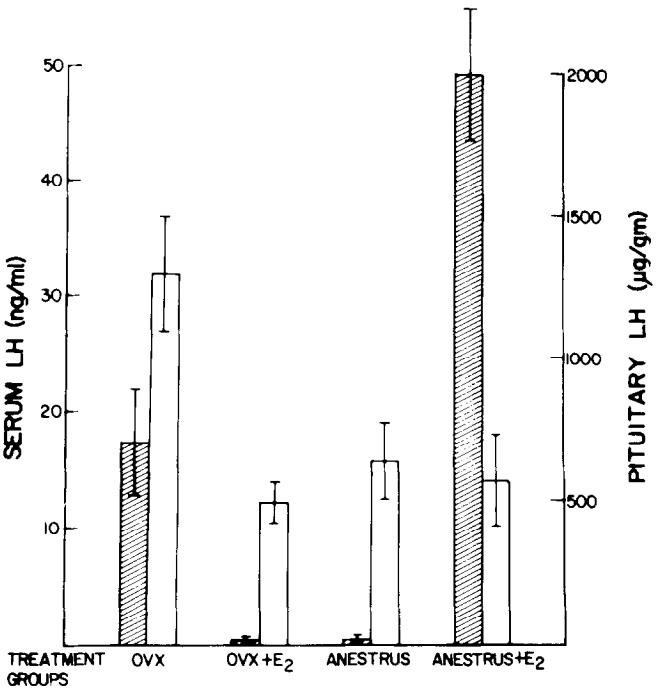

FIGURE 2. Serum and pituitary LH concentrations in treatment groups. The states of gonadal steroid feedback are described in Materials and Methods. Serum LH (▨) and pituitary LH (□) were determined by RIA and expressed as the mean ± SEM.

Quantitation of Beta Subunit Synthesis - The cell-free translation assays were standardized with respect to the amount of radioactivity incorporated into trichloroacetic acid - insoluble cpm/ug of RNA (~2-4 x 10^5 cpm/ug). All assays demonstrated an incorporation of ~10 fold greater than background (no exogenous RNA added) with subsaturating amounts of RNA. Immunoprecipitated PRL and GH did not vary among groups (Fig. 3). The amount of LH beta (Fig. 3), although less than the amounts of alpha, follows a similar pattern to that of alpha. For example, the amount of beta seen in the products translated from pituitary RNA of ovariectomized ewes is significantly higher than those values observed in products from the estradiol-treated ovariectomized group and the anestrous ewes. Additionally, the amount of immunoprecipitated beta subunit translated from RNAs from the intact anestrous ewes treated with estradiol is greater than those of the

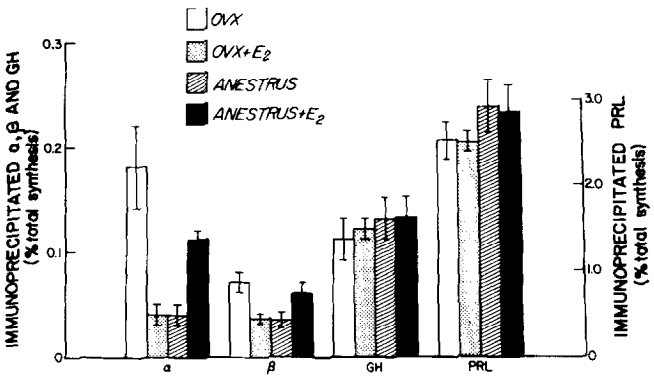

FIGURE 3. Amounts of immunoprecipitated pituitary proteins from cell-free assays encoded by ovine pituitary RNA. The amounts of the specific proteins are expressed as immunoprecipitated cpm/total cpm incorporated. The bars represent the mean ± SEM of at least 5 translation assays. The treatment groups are described in Materials and Methods.

negative feedback groups, but still slightly less than those values seen in the ovariectomized ewes.

DISCUSSION

The regulatory events involved in the expression of the gonadotropin subunit genes are complex and not well elucidated. However, recent studies have successfully correlated pituitary and serum LH amounts with pituitary gonadotropin subunit mRNAs, using various techniques such as cell-free translations (9-12) and cDNA hybridizations (13).

The results presented in this study provide evidence for negative and positive effects of estradiol on ovine LH subunit mRNA. Values for the ovariectomized group were reduced greater than 50% in animals under the influence of estradiol negative feedback, similar to that reported for FSH beta by Alexander and Miller (9). The amounts of beta subunit mRNA observed in anestrous animals treated with estradiol (positive feedback) approached those seen in ovariectomized ewes. Although serum LH was higher in these animals, pituitary LH amounts were similar to those observed in the negative feedback groups and less than that seen in the ovariectomized group.

Thus, it appears that an amount of LH is maintained in the pituitary despite acute changes in the serum amounts of the hormone, and that alpha and beta mRNAs contribute to maintaining these amounts since mRNA amounts of both subunits respond in these very different secretory stages. In castrated animals, where content is increased despite secretion being maintained relatively constant, mRNA levels are increased. The roles of GnRH and gonadal steroids in these processes of secretion and synthesis can not be delineated in these studies, although the effects of these regulators on transcription has been demonstrated (18-22).

The observed differences most likely represent changes in mRNA amounts, since incorporation was relatively constant for all RNAs. It is not known whether the immunoprecipitated amounts of these proteins are reflective of the absolute amounts of RNA since translation assays are an indirect measure; however, the relative ratios of these proteins are somewhat similar to those reported by Nilson et al (13).

These results correlate well with our data obtained for the alpha subunit using these animals models (10,11), suggesting that the synthesis of the individual subunits is separately but coordinately regulated. This separate regulation has also been shown for the subunits of the other pituitary glycoprotein hormone, TSH (23-24). Although it is not possible to delineate whether the synthesis of beta is rate-limiting, these data support those of Nilson et al (13) suggesting that this may not be the case since both subunits are under estradiol regulation. However, in addition, these studies have provided important new evidence for a positive estradiol effect on LH beta subunit mRNA amounts. This is especially relevant since the positive effect of this gonadal steroid is involved in the dramatic rise in serum LH during the preovulatory surge.

By correlating the amounts of subunit mRNAs with pituitary and serum LH concentrations in models such as these, the role of these effectors in reproductive cyclicity can be better delineated.

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