Regulation of β-chain mRNA of ovine follicle-stimulating hormone by 17β-estradiol*

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Summary

17β-Estradiol (E₂) rapidly and reversibly decreases the synthesis of follicle-stimulating hormone (FSH) in primary dispersed cell cultures of ovine pituitaries. Similarly, E₂ also causes a decrease in the messenger RNA for the beta subunit of ovine FSH (FSHβ-mRNA) as measured in an in vitro translation assay. These results are consistent with the concept that E₂ directly regulates either transcription of the FSHβ gene or processing of FSHβ-mRNA in sheep. Of additional interest is the observation that pituitary cultures from various species respond differently to E₂ in terms of FSH synthesis. For example, E₂ stimulates FSH synthesis in rat pituitary cultures, has no effect in similar rabbit cultures, and inhibits FSH synthesis in ovine cultures. Thus, a set of eukaryotic ‘mutants’ may exist to aid studies of the effect of E₂ on FSH synthesis and secretion.

Introduction

Physiological aspects of 17β-estradiol action on follicle-stimulating hormone

It is well known that gonadectomy in mammals (either castration or ovariectomy) often leads to a 5- to 20-fold increase in blood levels of follicle-stimulating hormone (FSH), a pituitary gonadotropin. Replacement of gonadal steroids, especially 17β-estradiol (E₂), dramatically reduces blood levels of FSH in such animals.

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Since cytoplasmic and nuclear receptors for E₂ occur in the pituitary (1, 2), it is possible that E₂ directly inhibits pituitary production of FSH. Several laboratories which study rat pituitary function have tried to demonstrate a direct inhibitory effect of E₂ on FSH production in vitro using dispersed cell cultures of rat pituitaries, but to date, no laboratory has succeeded (3–6). In fact, two reports indicate that E₂ actually stimulates FSH synthesis and/or secretion by 1- to 3-fold in rat cultures (6, 7).

Different results are obtained with ovine pituitary cultures, however. These cultures spontaneously produce more FSH than do rat cultures, and FSH is rapidly and reversibly inhibited with low levels of E₂ (10–80 pM; 2.7–22 pg/ml). Recently, it has been shown that FSH synthesis in pituitary cultures from the pig, cow, and human is also depressed by E₂ (see data below).

This review deals mainly with results obtained with ovine pituitaries, but evidence will be presented to show that there are significant species differences in how FSH is regulated in vitro. These differences seem important. First, they suggest that
different species have alternative mechanisms for regulating FSH. Knowledge of these mechanisms would be useful for reproductive physiologists. Second, altered FSH genes may ultimately be responsible for the observed differences in E2 action. Knowledge of these alterations may lead to greater insight into E2 action at the nuclear level.

The molecular biology of E2 action on FSH synthesis

It has been shown that E2 regulates the synthesis of several proteins by altering the rates of transcription for specific mRNAs (8–11). It does this in target cells by binding to cytoplasmic receptors which then migrate to the nucleus and, presumably, interact with chromatin in some fashion. In all cases documented to date, the effect of E2 has been to induce an increase in specific mRNAs. One might visualize the mechanism of E2-induced inhibition of FSH as analogous to the direct induction of new mRNAs, except that a specific gene product is shut off rather than turned on.

FSH is a glycoprotein composed of two nonidentical, noncovalently linked subunits (α and β). The α-subunit occurs also in two other pituitary glycoprotein hormones, luteinizing hormone and thyroid-stimulating hormone, both of which have their own hormone-specific β-subunits. Because the α-subunit is found in excess in pituitary tissue and serum, it is presently thought that FSH synthesis may be controlled specifically by the synthesis of its β-subunit (12). Furthermore, there is evidence that the common α- and all three β-subunits for the glycoprotein hormones are coded by separate mRNAs (13–17). Thus, it is possible for FSH synthesis to be controlled by regulating transcription of the gene for FSH β-subunit. Since E2 is thought to act at the level of transcription, initial efforts have focused on the effects of E2 on mRNA levels for the FSH β-subunit.

Materials and methods

Detailed methods have been published for most of the research reviewed in this article. What follows are specific references to these published procedures plus general considerations about the procedures which have not been published.

Pituitary source – human

The data on human pituitary cultures presented in Fig. 2 have never been published. Pituitaries for these cultures were obtained as autopsy samples generously supplied by the Department of Pathology, Duke University Medical Center, Durham, NC, through the courtesy of Kuo-Jang Kao. The pituitary donors were men, 50 to 60 years of age. Pituitaries were obtained 3 to 4 hr after death. The human pituitary cultures produced relatively small amounts of FSH (see Fig. 2), perhaps due to the long time between death of the patients and culturing of the pituitaries. Cultures were prepared as described (18).

Pituitary source – sheep

Pituitaries from more than 400 cross-bred ewes at all physiological states (estrus, anestrus, pregnant, lactating, ovarioctomized) have been tested in our laboratory. Every dispersed cell culture from every pituitary has synthesized relatively large amounts of FSH (100 to 200 ng/day/10⁶ cells). Such synthesis can be maintained at least 20 days (19). Pituitary cultures from castrate or intact male sheep do not maintain FSH secretion well, and FSH secretion declines to low levels within 14 days as it does in cultures from most other species tested (7).

It is convenient to have access to a small flock of sheep in the event that sheep need to be pretreated in vivo before sacrifice. Pituitaries can be obtained most economically, however, from a slaughter house. The slaughter house may be as far away as 8 hr travel distance, since ovine pituitary tissue is stable on ice in complete medium for at least 8 hr (see below).

Pituitary dispersion and culture

Detailed instructions for preparing ovine pituitary cultures are reported elsewhere (18), but some important considerations are presented below. Pituitaries should be taken from sheep within 5 to 30 min after slaughter, placed into ice-cold media 199 or Hanks balanced salt solution, and sliced into 1-mm thick slices at the laboratory. If laboratory slicing is to be delayed 1 to 8 hr, pituitaries should be sliced in a sterile glove bag at the abattoir and then