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Biosynthesis of gonadotropins *in vivo*

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Mini-review

Abstract. GnRH is potent stimulator of gonadotropin's α and β chains synthesis *in vivo*. Stimulation of LHβ gene transcription requires pulsatile GnRH administration but the transcription of α subunit can be stimulated independently of GnRH mode of administration. Castration increases whereas *in vivo* estradiol and testosterone replacement decreases the rate of gene transcription of pituitary gonadotropin subunits. Thyroid hormones can enhance or diminish the pituitary levels of LHβ and FSHβ subunit mRNAs in female rats. Inhibin, activin and follistatin were shown to be potent regulators of FSHβ gene expression.

Key words: luteinizing hormone, follicle stimulating hormone, gene expression regulation, gonadotropins, pituitary

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are pituitary glycoprotein hormones that regulate gonadal function including the production of sex-steroid hormones, maturation of ovarian follicles in the female and development of spermatocytes in the male. These hormones (gonadotropins) are essential for normal growth, development and reproduction. Each hormone is a dimer composed of two different carbohydrate containing subunits, termed alpha and beta, that are bound non-covalently. The α subunit is identical among glycoprotein hormones in the same species whereas the β subunits are substantially different for each hormone, and as such, specify biological activity. Gonadotropin subunits are encoded by separate genes (a single α gene and separate β subunit gene) that are located on separate chromosomes. Transcription of the rat α, LHβ and FSHβ subunits genes results in mature mRNAs of approximately 800, 700. and 1,700 nucleotides that encode the precursors of the α, LHβ and FSHβ subunits, respectively.

It has been proven that some factors influencing the release of gonadotropins like gonadotropin releasing hormone (GnRH), gonadal steroids, inhibin, activin, follistatin have also regulatory influence on LH/FSH synthesis. Recent progress in molecular biology indicates that the regulation of gonadotropin synthesis occurs at the level of gene expression.

Studies on primary cultures of dispersed pituitary cells showed that GnRH is a potent stimulator of gonadotropin's α and β chains synthesis (Khar et al. 1978, Starzec et al. 1986). It is now well established that stimulatory effect of GnRH on gonadotropin synthesis is reproduced in a nonadditive manner by either cyclic AMP and diacylglycerols, two intracellular mediators that can be generated by the interaction of GnRH with membrane receptors at the surface of the cell (Starzec et al. 1989a). Increased synthesis of α and LHβ mRNA were observed *in vitro* following pituitary cells stimulation by GnRH (Andrews et al. 1988, Starzec et al. 1988, 1989a). Dalkin et al. (1989) showed that the expression of gonadotropin genes depends on the frequency of GnRH stimulation. Also, Shupnik (1990) found striking effects of GnRH on gonadotropin subunit gene transcription, which were subunit and administration specific. Stimulation of LHβ gene transcription requires pulsatile GnRH administration and the transcription of α subunit can be stimulated independently of GnRH mode of administration. Lalloz et al. (1988) found that GnRH is required for enhanced luteinizing hormone subunit gene expression *in vivo*. These data suggest that gonadotropin subunit mRNAs are regulated primarily by GnRH and not by direct negative estrogen feedback on the pituitary. Hamernik et al. (1986) and Mercer et al. (1988) reported that in ovariectomized ewes hypothalamic-pituitary disconnection resulted in decreased synthesis of LHβ, FSHβ and α-subunit mRNAs, whereas pulses of GnRH restored gonadotropin subunit mRNAs to the levels observed in ovariectomized animals.

Castration increases whereas *in vivo* estradiol or testosterone replacement decreases the rate of gene trasncription of pituitary gonadotropin subunits in rat and sheep (Alexander and Miller 1982, Counis et al. 1983, Landefeld and Kepa 1984). Progesterone was found to decrease pituitary gonadotropin mRNA (Corbani et al. 1990). Both in male and in female rats, estrogens negatively regulate all three gonadotropin subunit mRNA levels, whereas androgens negatively regulate LHβ and α subunit, but fail to suppress FSHβ mRNAs (Wierman et al. 1988, Counis and Jutisz 1991).

Regulatory factors other than GnRH and steroids also participate in direct or indirect modulation of pituitary gonadotropin gene expression. It was reported that thyroid hormones can enhance or diminish the pituitary levels of LHβ- and FSHβ-subunit mRNAs in female rats and those effects were dependent on the dose of injected triiodothyronine or thyroxine as well as on the presence or absence of gonads in the rat (Lerrant et al. 1988). Also such peptides as inhibin, activin and follistatin were shown to be potent regulators of FSHβ gene expression (Attardi et al. 1989, Carroll et al. 1989).

Studies on synthesis and secretion of FSH in ewes have shown a balance between the stimulatory influence of GnRH and activin and the inhibitory

effects of estradiol and inhibin on those processes (McNeilly et al. 1995). Acting directly at the level of pituitary both inhibin and estradiol have caused a decrease of FSHβ subunit gene expression by reducing as well as transcription rate and mRNA stability in the cytoplasm. Also activin exerts important influence on gonadotropin synthesis and release. It was observed that recombinat activin A subcutaneous injections to the male rats resulted in 60% increased both the release and synthesis of FSH. When activin was administered to the ovariectomized, estradiol treated female rats the 2.5 fold enhanced FSH gene expression in their pituitaries was noticed after 5 h.

Inhibin and activin subunits have been colocalised within gonadotrope cells in the pituitary, so it cannot be excluded that these peptides can exert their autocrine regulatory effect directly in the pituitary (Mercer 1990). In our experiments, we have studied the effect of pulsatile injection of GnRH into 3rd cerebral ventricle of cycling or ovariectomized female rat on the expression of gonadotropin genes *in vivo* (Kochman et al., unpublished data). Cannules were implanted into the third cerebral ventricle using stereotaxic apparatus and an infusions by micro-pump were each 15, 30 or 60 min intervals (10 μl volume) during 5 h mRNA for subunits α and LHβ, FSHβ and β actin were measured. Obtained data have shown that after the ovariectomy a pulsatile injection of GnRH every 30 min through 5 h have caused an increase of pituitary mRNA level for LHβ (18%) as well as for FSHβ (25%); whereas after an infusion every 60 min during 5 h such stimulatory effect was much more expressed both for LHβ (62%) and FSHβ (85%).

When GnRH was injected into the 3rd ventricle of proestrus female it stimulated the biosynthesis of mRNA for α subunit (46%) and LHβ (56%) while in estrus this stimulation was 48% and 64%, respectively. These results support the conception that primary action of GnRH occurs through its stimulatory effect on gonadotropin biosynthesis, however the influence of GnRH action on gonadotropin subunits glycosylation cannot be excluded. Further, we examined the influence of naloxone and β-endorphin infusions on the gonadotropin mRNA subunits synthesis. β-endorphin inhibited significatively the mRNA for LHβ (-57%) and subunit α (-26%). but the naloxone had no shown any effect on the pituitary mRNA formation of gonadotropins. Such β-endorphin's inhibitory effect on mRNA gonadotropins synthesis (as well as on their release) can be ascribed to its inhibitory effect on GnRH release at the level of hypothalamus. On the other hand, lack of the evident stimulation of gonadotropins' mRNAs synthesis observed after naloxone infusions can be a result of the low level of endogenous β-endorphin in the hypothalamus in the ovariectomized rats. Domański et al. (1991) reported that β-endorphin content in ewe's hypothalamus is very low during the period of GnRH release. Also, Van Vugt et al. (1982) described the increase in the LH secretion after gonadectomy as a consequence of diminishing inhibitory action of opioids after removal of gonads. Pinilla et al. (1994) did not observed any change of LH secretion in ovariectomized rat after naloxone administration.

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