Korean J. of Pharmacology
Vol. 30, No. 2, pp. 19-28, 1994

Regulation of Luteinizing Hormone Release and Subunit mRNA by GnRH and Ovarian Steroids in Cultured Anterior Pituitary Cells

Changmee Kim*, Il Sun Park* and Kyungza Ryu

Department of Pharmacology and Endocrine Laboratory, Yonsei University, College of Medicine, Seoul, Korea*

ABSTRACT

The effects of gonadoropin-releasing hormone (GnRH) and ovarian steroid hormones on the release of luteinizing hormone (LH) and its subunit mRNA levels were investigated in anterior pituitary cells in culture. LH concentration was measured by a specific radioimmunoassay and mRNA levels of α and LHβ subunits by RNA slot blot hybridization assay. GnRH stimulated LH release in a dose-dependent manner from cultured pituitary cells. However, the basal LH release in the absence of GnRH was not changed during the course of 24h culture, strongly suggesting that release of LH is directly controlled by GnRH. The treatment of the pituitary cells with GnRH increased LHβ subunit mRNA levels in a dose-dependent manner, reaching the maximum with 2×10^{-10} M GnRH while no significant increase in α subunit mRNA levels was observed after GnRH treatment. Estradiol did not augment GnRH-induced LH release while progesterone augmented GnRH-induced LH release in a dose-dependent manner at the level of pituitary. However, estradiol and progesterone increased basal and GnRH-induced LHβ subunit mRNA levels in a dose-dependent manner. The treatment of estrogen antagonist, LY117018 blocked the effect of estradiol on GnRH-induced LHβ subunit mRNA levels in a dose-dependent manner while progesterone antagonist, RU486 tended to block the effect of progesterone on GnRH-induced LHβ subunit mRNA levels. It is therefore suggested that GnRH plays a major role in LH release and subunit biosynthesis by influencing the steady state LHβ subunit mRNA leves and ovarian steroid hormones modulate subunit biosynthesis via directly acting on pituitary gonadotropes.

Key Words: LHβ subunit, GnRH, Estrogen, Progesterone, Pituitary primary cell culture

INTRODUCTION

Luteinizing hormone (LH) is a pituitary gonadotropin that plays an important role in sexual development and function, mainly through its influence on gonadal steroidogenesis and gametogenesis. LH, like follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), is a glycoprotein hormone and composed of α and β subunits which are translated from distinct mRNAs encoded by different genes on different chromosomes (Chin, 1986). Within a species, structure of α subunit of glycoprotein hormones is nearly identical and an uniqueness of β subunit

This work was supported by the Ministry of Education (1990 GE program)

confers the biological specificity to each hormone (Pierce and Parsons, 1981).

The secretion of LH is known to be regulated by the hypothalamic GnRH and the gonadal steroid hormones. LH secretion follows two distinct and functionally separate modes, the preovulatory and the tonic releases, and these are regulated by positive and negative feedback actions of ovarian steroid hormones respectively. GnRH appears to exert stimulatory effects on cellular responses involved in LH secretion, receptor upregulation (Papavasiliou *et al.*, 1986b; Naor, 1990), subunit biosynthesis (Starzec *et al.*, 1986), and subunit glycosylation (Krummen and Baldwin, 1988). Gonadal steroid hormones play a crucial role in LH secretion by acting at the hypothalamic level, thereby modulating the pattern of GnRH secretion, and by directly acting at the pituitary level. (Frawley and Neil, 1984; Saade *et al.*, 1989). However, the mechanisms by which GnRH and ovarian steroids regulate LH synthesis as well as release and the interaction between these factors are not clearly demonstrated.

During the past years, the availability of cDNA clones for α and LHβ subunits has allowed investigation for the regulation of LH subunit gene expression by determining the steady state mRNA levels.

Our previous studies have shown that in *in vivo* paradigm such as ovariectomy and ovarian steroid hormone replacement, the circulating levels of LH and FSH generally reflect the steady state mRNA levels of subunits and ovarian steroid hormones negatively regulate LH and FSH synthesis at the pretranslational level by influencing the steady state mRNA levels of α, LHβ and FSHβ subunits (Ryu *et al.*, 1989; Lee, 1990; Kim *et al.*, 1993).

The present study was therefore attempted to determine whether the release of LH is directly coupled with LHβ subunit gene expression and whether GnRH and ovarian steroids directly modulate LHβ subunit gene expression at the pituitary level. The studies described herein focus on the effects of GnRH on the synthesis and release of LH and their modulation by ovarian steroid hormones by determining the steady state levels of LHβ mRNA in static culture of anterior pituitary cells in rats.

MATERIALS AND METHODS

Primary culture of anterior pituitary cells

Adult female Sprague Dawley rats (200~250 g) were decapitated and anterior pituitary glands were immediately removed and sliced. Pituitary fragments were incubated in Spinner's Minimum Essential Medium (S-MEM) with trypsin (0.4%) and DNase (10 ug/ gland) at 4℃ for 1 hr and then at 37℃ for 30 min. Incubates were centrifuged at 400 xg for 5 min and the pellet was suspended in S-MEM with trypsin inhibitor (25 ug/ml), repeating gentle aspiration and expulsion 40 times with Pasteur pipette. The cell suspension was passed through Swinnex filter (74-, 20-, and 15 um nylon mesh). The density of cell was adjusted to 2×10^6 cells/ well in 2 ml Eagle's Minimum Essential Medium (E-MEM) with penicillin (100 u/ml), streptomycin (100 ug/ml), 10 % horse serum and 2.5 % fetal calf serum. All sera were dextran-charcoal stripped for the removal of residual steroids. Cells were incubated in 5 % CO_2 at 37℃ for 2 days and fresh medium was replaced for each experiment. After treatment of each reagent, medium was stored at −20℃ for LH quantitation and cells were harvested with policemen and stored in liquid nitrogen for mRNA determination. GnRH, estradiol and progesterone were dissolved in absolute ethanol. The final concentration of ethanol was less than 0.1 %.

Radioimmunoassay (RIA) for rat LH (rLH)

LH concentrations in culture medium and cultured cells were determined by RIA using rLH standard (rLH-RP-3) and antibody (anti-rLH-s-10) provided by NIDDK (National Institute of Diabetes & Digestive & Kidney Disease, U.S.A.). rLH was iodinated with ^{125}I by chloramine-T method. Antibody was used at a final dilution of 1: 40,000. The sensitivity of rLH assay was 0.1 ng/ml and interassay coefficient of variation was 9%.

Determination of α and LHβ subunit mRNA

Probe preparation: Rat α and LHβ cDNA probes were isolated from recombinant plasmids (pBR322-α and pGEM 3-LHβ), kindly provided by

Dr. R. Maurer (University of Iowa, U.S.A.) with pst 1 digestion. γ-actin cDNA probe was used as an internal standard. γ-actin cDNA was isolated from Okayama-Berg cDNA cloning vector with Bam H1 digestion. Each cDNA was labelled by random primer labelling method with α-^{32}P-dCTP.

RNA preparation: Total RNA was isolated from cultured pituitary cells (2×10^6 cells) by Nonidet P-40 method (Papavasilliou *et al.*, 1986a). Briefly, pituitary cells were homogenized by glass homogenizer containing 220 ul homogenization buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.5% Nonidet P-40). The homogenate was transferred to a sterile tube and added proteinase K (0.01%, W/V). After incubation (37℃, 30 min), sample was extracted with phenol/chloroform. RNA was precipitated overnight at −20℃ with 0.1 volume of 0.3 M sodium acetate (pH 5.4) and absolute ethanol (2.5~3 vol.). Total RNA was then recovered by centrifugation (13,000 xg) at 4℃ for 30 min and dissolved in D.W.

Slot blot hybridization: RNA (2~3 ug) in 50 ul D.W. was transferred to a sterile tube containing 30 ul of 20x SSC and 20 ul of 37% formaldehyde. The mixture was then incubated at 65℃ for 10 min, and chilled on ice. RNA was blotted to Nytran membrane (0.45 um pore size) using slot blot apparatus (Manifold, Schleicher & Schuell. W. Germany). Wells were rinsed with 15x SSC and then baked in vacuum oven at 80℃ for 2 hr.

Hybridization and autoradiography: The hybridization procedure was similar to that previously described by Papavasilliou *et al.* (1986a). Briefly, Nytran membrane was prehybridized in 50 % formamide, 5x SSPE, 5x Denhardt's solution, 0.1 % SDS and 2 mg heat-denatured salmon sperm DNA at 42℃ for 3 hr. After prehybridization, the blots were hybridized with ^{32}P-labelled probe at 42℃ for 18hr. The blots were washed three times with 2x SSC and 0.1 % SDS at room temperature and once with 0.1x SSC and 0.1 % SDS at 50℃. The slot density in autoradiogram was assessed by densitometric analysis. The integrated peak area of each slot was expressed as arbitrary densitometric unit (ADU).

Statistical analysis

The differences between experimental groups were analyzed by one-way ANOVA with t-test and P values less than 0.05 were considered significant.

RESULTS

Effect of GnRH on the LH release and LH subunit mRNA levels in anterior pituitary cells

As shown in Fig. 1A and 2, all doses of GnRH (5

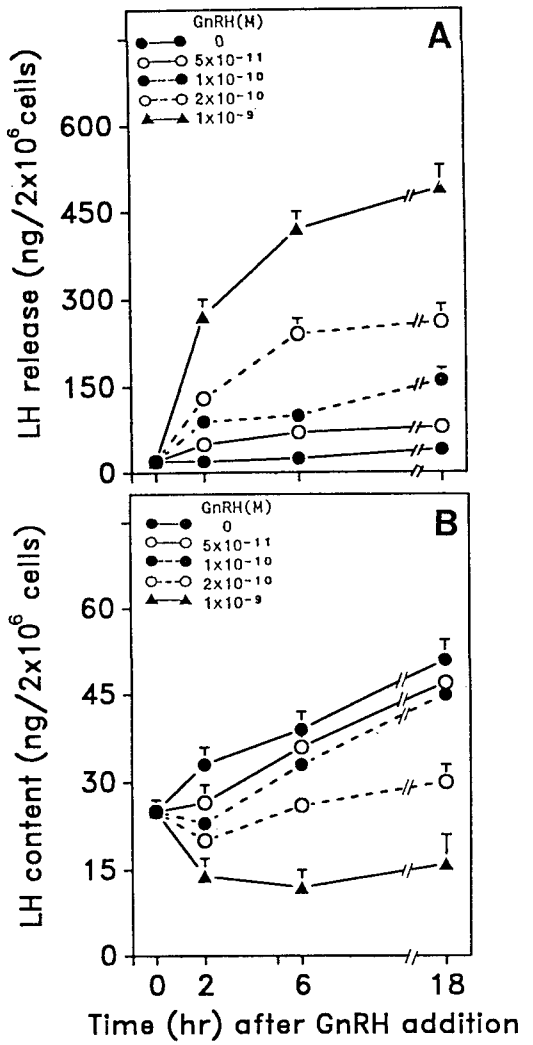

Fig. 1. Effect of GnRH on the LH release (A) and contents (B) in anterior pituitary cells in culture. Each point represents the mean±S.E. of repeated experiments (n=3 to 4).

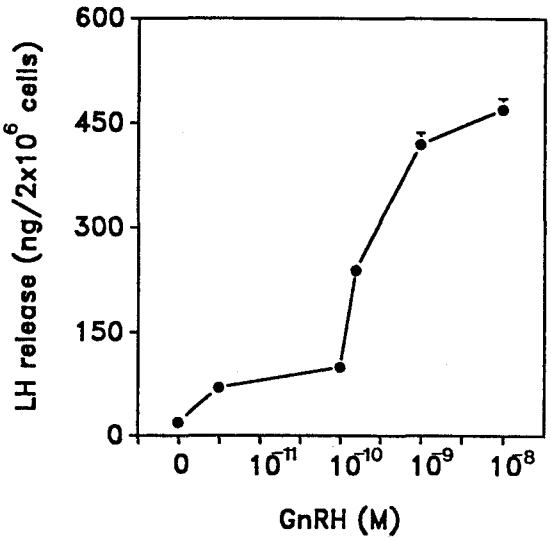

Fig. 2. Dose-response analysis of GnRH stimulation of LH release from anterior pituitary cells in culture. Each point represents the mean±S.E. of repeated experiments (n=3 to 4).
*P<0.05, **P<0.01; compared with control group.

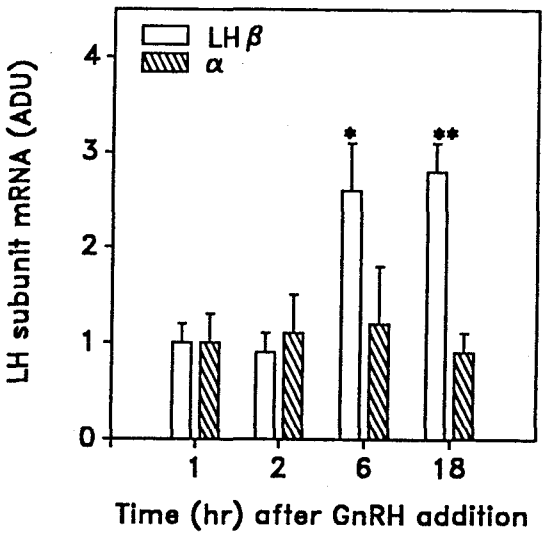

Fig. 3. Time course of changes in α and LHβ subunit mRNA levels after GnRH treatment in anterior pituitary cells in culture. Each point represents the mean±S.E. of repeated experiments (n=3 to 4).
*P<0.05, **P<0.01; compared with control group.

$\times 10^{-11} \sim 1 \times 10^{-9}$ M) stimulated LH release from the pituitary cells. The LH response to GnRH was time-and dose-dependent, the release being sharply increased during the first 2 hrs after GnRH treatment. In the absence of GnRH, LH release was not changed with time. In the presence of GnRH, LH contents in cultured anterior pituitary cell were negatively correlated with LH release (Fig. 1B). However, LH contents in the pituitary cells tended to increase with time after GnRH addition except the highest dose of GnRH, which induced maximum LH release, suggesting a time-dependent accumulation of newly produced LH after rapid release.

To investigate whether GnRH increased LH subunit mRNA levels in static culture of anterior pituitary cells, 2×10^{-10} M of GnRH was treated. As shown in Fig. 3, the treatment of pituitary cells with GnRH increased LHβ subunit mRNA levles in a time-dependent manner while α subunit mRNA levels were not changed. GnRH also increased LHβ subunit mRNA levels in a dose-dependent manner, reaching the maximum with $2 \times$

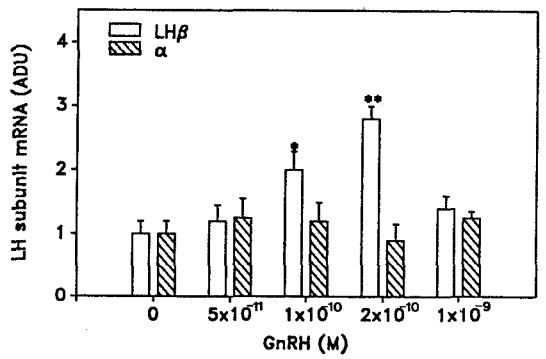

Fig. 4. Effect of GnRH on α and LHβ subunit mRNA levels in anterior pituitary cells in culture. Each point represents the mean±S.E. of repeated experiments (n=3 to 4).
*P<0.05, **P<0.01; compared with control group.

10^{-10} M GnRH while no significant increase in α subunit mRNA levels was observed (Fig. 4).

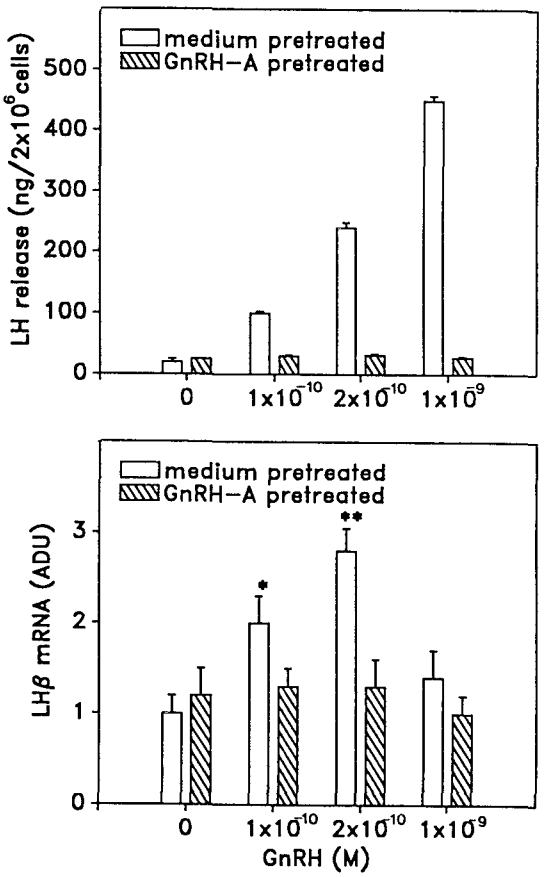

Fig. 5. Effect of GnRH agonist ([d-Ser(But)6-Pro9]-GnRH: Buserelin) on GnRH-induced LH release and LHβ subunit mRNA levels in rat anterior pituitary cells. Anterior pituitary cells pretreated for 24 hrs with/without 10^{-7} M Buserelin were further incubated with 2×10^{-10} M GnRH. Each bar represents the mean ±S.E. of repeated experiments (n=3 to 4). GnRH-A; GnRH agonist. *P<0.05, **P<0.01; compared with control group.

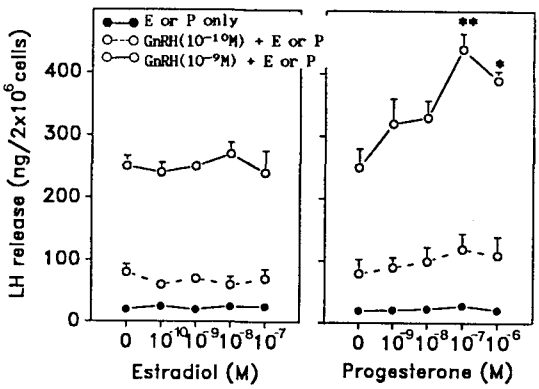

Fig. 6. Effects of estradiol and progesterone on GnRH-stimulated LH release from rat anterior pituitary cells in culture. Each point represents the mean ±S.E. of repeated experiments (n=3 to 4). E, estradiol; P, progesterone
*P<0.05, **P<0.01; compared with GnRH-only group.

Effect of GnRH agonist on the LH release and LHβ subunit mRNA levels in anterior pituitary cells

To confirm the effect of GnRH on the LH release and LHβ subunit mRNA levels, pituitary cells were pretreated for 24 hrs with/or without 10^{-7} M GnRH agonist, D-[Ser(But)^6-Pro^9]-GnRH (Busere-lin) and further incubated with GnRH (2×10^{-10} M) for 18 hrs. The dose of Buserelin treated was supposed to induce down-regulation of GnRH receptors in gonadotropes. The pretreatment of Buserelin completely suppressed the stimulatory effect of GnRH on LH release and LHβ subunit mRNA levels (Fig. 5).

Effects of ovarian steroids on LH release in cultured anterior pituitary cells

To investigate the effects of ovarian steroids on the LH release, anterior pituitary cells were treated with estradiol or progesterone for 18 hrs. As shown in Fig. 6, the treatment of estradiol- or progesterone-only did not stimulate LH release from pituitary cells.

Moreover, GnRH-induced LH release from pituitary cells was not modulated by the treatment of various doses of estradiol. However, the treatment of progesterone augmented GnRH-induced LH release in a dose-dependent manner (Fig. 6). To confirm the effect of progesterone on GnRH-stimulated LH release, pituitary cells were treated with progesterone and progesterone antagonist, RU486. RU486 blocked the stimulatory effect of progesterone on GnRH-induced LH release in a dose-dependent manner (Fig. 7).

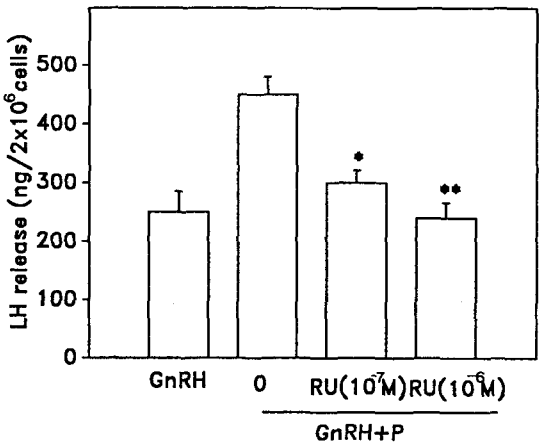

Fig. 7. Effects of progesterone and progesterone antagonist (RU486) on GnRH-stimulated LH release from anterior pituitary cells in culture. Each point represents the mean±S.E. of repeated experiments (n=4 to 5).
GnRH+P, GnRH+progesterone; RU, RU486
*P<0.05, **P<0.01; compared with GnRH+P group.

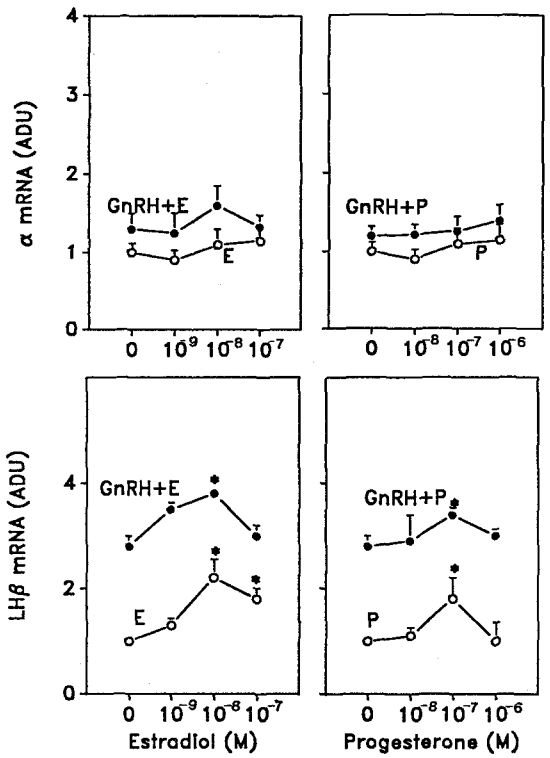

Fig. 8. Effects of estradiol and progesterone on α and LHβ subunit mRNA levels in anterior pituitary cells in culture.
Each points represents the mean±S.E. of repeated experiments (n=5). E, estradiol; P, progesterone
*P<0.05; compared with control group.

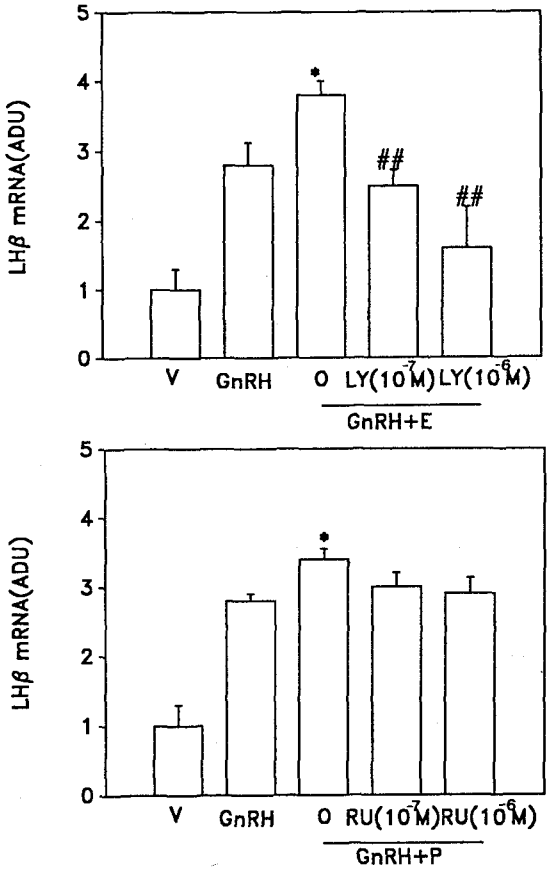

Fig. 9. Effects of ovarian steroids and their antagonists on GnRH-stimulated LHβ subunit mRNA levels in anterior pituitary cells in culture. Each bar represents the mean±S.E. of repeated experiments (n=4 to 5); LY, LY117018. RU, RU486
*P<0.05; compared with GnRH+P group.
##P<0.01; compared with GnRH+E group.

Effects of ovarian steroids on LH subunit mRNA levels in cultured anterior pituitary cells

As shown in Fig. 8, the treatment of estradiol or progesterone with/or without GnRH did not stimulate α subunit mRNA levels in anterior pituitary cells. But the treatment of estradiol- or progesterone-only stimulated basal LHβ subunit mRNA levels and enhanced GnRH-induced LHβ subunit mRNA levels in a dose-dependent manner. To confirm the effect of estradiol or progesterone on GnRH-stimulated LHβ subunit mRNA levels, pituitary cells were incubated with estradiol and estrogen antagonist, LY117018, or progesterone and progesterone antagonist, RU486. The treatment of LY117018 blocked the effect of estradiol on GnRH-induced LHβ subunit mRNA levels in a dose-dependent manner while effect of RU486 was not statistically significant (Fig. 9).

DISCUSSION

The present study indicates that GnRH plays a major role in LHβ subunit biosynthesis as well as LH release, by influencing the steady state levels of LHβ subunit mRNA levels in static culture of anterior pituitary cells.

Although *in vivo* studies showed that GnRH regulates gonadotropin subunit gene expression, *in vivo* studies indicated divergence in gonadotropin subunit mRNA response to GnRH, with some disagreements among investigators (Andrews *et al.*, 1988; Salton *et al.*, 1988). Since evidence suggests that not only the presence of GnRH but also the dose and frequency of administration plays a critical role in determining its effect on gonadotropin subunit gene expression *in vivo* (Haisenleder *et al.*, 1988; Dalkin *et al.*, 1989), pituitary perifusion system has the advantage of permitting exogenous signals to be applied in a pulsatile fashion (Shupnik, 1990; Weiss and Jameson, 1993). However, static culture system of pituitary cells used in our study has been useful as an *in vitro* system, since nonpulsatile treatment of low concentrations of GnRH appeared to stimulate LH synthesis as well as release in static culture (Andrews *et al.*, 1988). It has been reported that low doses of GnRH (10^{-10} M) increased LHβ subunit mRNA levels in cultured anterior pituitary cells of immature rats (Andrews *et al.*, 1988). In the present study using adult rats, the treatment of pituitary cells with GnRH also increased LHβ subunit mRNA levels in a dose-dependent manner, reaching the maximum with 2×10^{-10}M. The ability of GnRH to stimulate LHβ subunit mRNA levels in static cultures provides a simplified system for further studies of GnRH action on LH subunit gene expression.

Several lines of evidence indicate that synthesis of LHβ subunit is the rate-limiting step in the biosynthesis of LH (Papavasiliou *et al.*, 1986b; Leung *et al.*, 1988; Kim *et al.*, 1993).

One of points of the present *in vitro* study is that ovarian steroids positively regulate gene expression of LHβ subunit at the pituitary level. On the other hand, our previous *in vivo* studies have shown that ovarian steroids negatively regulate LH and FSH synthesis at the pretranslational level by influencing the steady state mRNA levels of α, LHβ and FSHβ subunits (Ryu *et al.*, 1989; Lee, 1990; Kim *et al.*, 1993). In mammals, ovarian steroids exert both negative and positive feedback effects on gonadotropin secretion and synthesis. Negative feedback action of estrogen seemed to be operated only at the level of hypothalamus. However, the positive feedback effect of estrogen include actions at the hypothalamus to increase the synthesis (Radovick *et al.*, 1991) as well as secretion of GnRH (Karsch, 1987) and at the pituitary to enhance its responsiveness to GnRH (Wun, 1987; Kaiser *et al.*, 1993). In our results, estrogen significantly increased GnRH receptors in gonadotropes (data not shown). Several authors demonstrated that estrogen augmented GnRH-induced LH release from rat pituitary (Frawley and Neil, 1984; Emons *et al.*, 1989; Kotsuji *et al.*, 1988; Baldwin *et al.*, 1991) or rhesus monkeys (Frawley and Neil, 1984). Therefore it is assumed that ovarian steroids might modulate pituitary responsiveness to GnRH or might directly stimulate LHβ subunit gene expression, thereby increasing the steady state levels of LHβ subunit mRNA.

The change in the steady state level of mRNA might reflect regulation at the level of either gene transcription or mRNA stability. Shupnik *et al.* (1989) reported that estradiol stimulated LHβ

transcription rate 2- to 3- folds, but not α subunit, in short term cultures of pituitary fragments. Indeed, a finding indicates that an estrogen responsive element (ERE) domain is present in the 5'-flanking region of LHβ subunit gene in rats (Shupnik *et al.*, 1988). Hence, it might be possible that estrogen directly regulates LHβ subunit biosynthesis at the transcriptional level. Moreover, steroid hormones have been known to control the degradation of specific mRNA (Nielson and Shapiro, 1990). Estrogen regulated the stability of apoVL II mRNA (Cochrane and Deely, 1988) and vitellogenin mRNA (Gordon *et al.*, 1988). Recently, our laboratory found that estradiol and progesterone modulated LHβ subunit mRNA levels, in part by increasing mRNA stability (unpublished data). Therefore, the changes in the steady state LHβ subunit mRNA levels by GnRH and ovarian steroids in the present study might be due to the changes in transcription of LHβ subunit and/or LHβ subunit mRNA stability.

From the present study, we conclude that GnRH plays a major role in LH release and synthesis by influencing the steady state LHβ mRNA levels and ovarian steroid hormones regulate LH synthesis as well as release partly by acting at the pituitary level.

REFERENCES

Andrews WV, RA Maurer and PM Conn: *Stimulation of rat luteinizing hormone β mRNA levels by gonadotropin releasing hormone. J Biol Chem 263: 13755-13761, 1988*

Baldwin DM, PS Srivastava and LA Krummen: *Differential actions of corticosterone on luteinizing hormone and follicle stimulating hormone biosynthesis and release in cultured rat anterior pituitary cells: Interactions with estradiol. Bio Reprod 44: 1040-1050, 1991*

Chin WW: *Glycoprotein hormone genes In; Habener JF(ed) Genes Encoding Hormones and Regulatory Peptide. Human Press, Clifton, NJ, pp137-172, 1986*

Cochrane AW and RG Deely: *Estrogen-dependent activation of the avian very low density apolipoprotein II and vitellogenin genes. Transient alterations in mRNA polyadenylation and stability during induction. J Mol Biol 203: 555-567, 1988*

Dalkin AC, DJ Haisenleder, GA Ortolano TR, Ellis and JC Marshall: *The frequncy of gonadotropin-releasing hormone stimulation differentially regulates gonado-tropin subunit messenger ribonucleic acid expression. Endocrinology 125: 917-17, 1989*

Emons G, O Ortmann, U Fingscheidt and R Knuppen: *Short-term effects of oestradiol and 4-hydroxyoestradiol on gonadotropin-releasing hormone induced luteinizing hormone secretion by rat pituitary cells in culture. Acta Endocrinol (Copenh) 111: 312-320, 1986*

Frawley S and JD Neill: *Biphasic effects of estrogen on gonadotropin-releasing hormone-induced luteinizing hormone release in monolayer cultrues of rat and monkey pituitary cells. Endocrinology 114: 659-663, 1984*

Gordon DA, GS Shellness, M Nicosia and DL Williams: *Estrogen-induced destabilization of yolk precursor protein mRNAs in avian liver. J Biol Chem 263: 2625-2631, 1988*

Haisenleder DJ, JA Katt, GA Ortolano, MR El-Gewelym JA Duncann, C Dee and JC Marshall; *Influence of gonadotropin-releasing hormone pulse amplitude, frequency and treatment duration of the regulation of luteinizing hormone (LH) subunit messenger ribonucleic acid expression Mol Endocrinol 2: 338, 1988*

Kaiser UB, A Jakubowiak, A Steinberger and WW Chin: *Regulation of rat pituiary gonadotropin-releasing hormone receptor mRNA levels in vivo and in vitro. Endocrinology 133: 931-934, 1993*

Karsch FJ: *Central actions of ovarian steroid in the feedback regulation of pulsatile secretion of luteinizing hormone. Ann Rev Physiol 49: 1349-1377, 1987*

Kim F, SJ Winters, B Attardi, HS Keeping, H Oshima and P Troen: *Effects of gonadal steroids on gonadotropin secretion in males: Studies with perifused rat pituitary cells. Endocrinology 123: 2683-2689, 1988*

Krummen LA and DM Baldwin :*Regulation of luteinizing hormone subunit biosynthesis in cultured male anterior pituitary cells: Effects of gonadotropin-releasing hormone and testosterone. Endocrinology 123: 1868-1878, 1988*

Naor ZVI: *Signal transduction mechanisms of Ca Mobilizing hormones: The case of gonadotropin-releasing hormone. Endoc Rev 11: 326-353, 1990*

Nielson DA and DJ Shapiro: *Insights into hormonal control of messenger RNA stability. Mol Endocrinol 4: 953-957, 1990*

Lee BS:*Regulation of FSH subunit synthesis in anterior pituitary gland in rat. Ph.D Thesis, Yonsei Univ, 1990*

Leung K, AH Daynard, BP Negrini, KE Kim, RA

Maurer and TD Landerfeld: *Differential regulation of gonadotropin subunit messenger ribuncleic acid by gonadotropin-releasing hormone pulse frequency in ewes. Mol Endocrinol 1: 724-731, 1987*

Oppenheim DS, H Bikkal, WF Crowley and A Kilbanski: *Effects of chronic GnRH analogue administration on gonadotropin and α subunit secretion in post-menopausal women. Clin Endocrinol 36: 559-564, 1992*

Papavasiliou SS, S Zmeili, L, Herbon, J Duncan-Weldon, JC Marshall and TD Landefeld : *α And luteinizing hormone β messenger ribonucleic acid (RNA) of male and remale rats after castrationL Quantitation using an optimized RNA dot blot hybridization assay. Endocrinology 119: 619, 1986a*

Papavasiliou SS, S Zmeili, L, S Khoury, TD Landefeld, WW Chin and JC Marshall: *Gonadotropin-releasing hormone differentially regulates expression of the genes for luteinizing hormone α and β subunits in male rats. Proc Natl Acad Sci USA 83: 4206-4209, 1986b*

Pierce JG and TF Parsons: *Glycoproteins: structure and function. Annu Rev Biochem 50: 465-495, 1981*

Radovick S., CM Ticknor, Y Nakayama, AC Notides, A Rahman, BD Weintraub, GB Cutler and FE Wondisford: *Evidence for direct estrogen regulation of the human gonadotropin-releasing hormone gene. J Clin Invest 88: 1649-1655, 1991*

Ryu K, C Kim, M Yoon and SS Hong: *Regulation of α subunit and LHβ subunit mRNA by estradiol and morphine in ovariectomized in rats. Proc Mol Bio Genet 4: 273-279, 1989*

Saade G, DR London and RN Clayton :*The interaction of gonadotropin-releasing hormone and estradiol on luteinizing hormone and prolactin gene expression in female hypogonadal (hpg) mice. Endocinology 124: 2149, 1989*

Salton SRJ, M Blum, JA Jonassen, RN Clayton and JL Roberts: *Stimulation of pituitary luteinizing hormone secretion by gonadotropin-releasing hormone is not coupled to β luteinizing hormone gene transcription. Mol Endocrinol 2: 1033, 1988*

Shupnik MA: *Effects of gonadotropin-releasing hormone on rat gonadotropn gene transcription in vitro: Requirement for pulsatile administration for luteinizing hormone β gene stimulation. Mol Endocrinol 4: 1444-1450, 1990*

Shupnik MA, SD Gharib and WW Chin: *Divergent effects of estradiol on gonadotropin gene transcription in pituitary fragments. Mol Endocrinol 3: 474-480, 1989*

Shupnik MA, CM Wienmann, AC Notides and WW Chin: *An upstream region of the rat luteinizing hormone β gene binds estrogen receptor and confers estrogen responsiveness. J Biol Chem 254: 80-86, 1988*

Starzec A, R Counis and M Justiz: *GnRH stimulates the synthesis of the polypeptide chains of LH. Endocrinology 119: 561-567, 1986*

Weiss J, JL Jameson: *Perifused pituitary cells as a model for studies of gonadotropin biosynthesis and secretion. Trends Eno Metab 8: 265-270, 1993*

Wun WS, and IH Thorneycroft: *Estradiol positive feedback on the rat anterior pituitary gland in vivo. Mol Cell Endocrinol 54: 165-169, 1987*

=국문초록=

흰쥐 뇌하수체전엽 배양세포에서 GnRH 및 난소호르몬에 의한 LHβ subunit 유전자 발현 조절에 관한 연구

연세대학교 의과대학 약리학교실, 내분비연구실*

김 창 미* · 박 일 선* · 유 경 자

흰쥐의 뇌하수체 전엽배양세포에 gonadotropin-releasing hormone (GnRH)을 처리하였을 때 시간이 경과함에 따라 GnRH농도에 비례하여 luteinizing hormone (LH)의 분비가 증가하였으며, 2시간까지 급격하게 증가하였다. 또한 GnRH를 처리하였을때 α subunit mRNA의 농도는 증가하지 않았으나 LHβ subunit mRNA의 농도는 GnRH 농도에 비례하여 증가하였으며, GnRH 처리후 6시간 이후부터 유의하게 증가하였다. 특히 최종농도가 2×10^{-10} M이 되도록 GnRH를 처리하였을때 LHβ subunit mRNA 농도가 2.7배 정도 최대로 증가하였다. 또한 estradiol을 단독으로 또는 GnRH와 동시에 처리하였을때 LH분비가 증가하지 않았으나 progesterone을 GnRH와 동시에 처리하였을때 LH분비가 유의하게 증가하였다. 또한 LHβ subunit mRNA의 농도는 estradiol 및 progesterone을 단독으로 또는 GnRH와 동시에 처리하였을때 난소호르몬 농도에 의존적으로 LHβ subunit mRNA의 농도가 증가하였다. Estradiol에 의한 LHβ subunit mRNA의 증가양상은 estrogen 길항제인 LY117018에 의하여 유의하게 감소하였다.

이러한 결과로 보아 GnRH는 steady state LHβ subunit mRNA 농도에 영향을 미치므로써 LH 분비 및 LH subunit 생합성을 조절하며 난소호르몬은 뇌하수체에 직접 작용하여 LH분비 및 LH subunit 생합성에 영향을 주는 것으로 보인다.