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Regulation of LHβ subunit mRNA by Ovarian Steroid in Ovariectomized Rats

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ABSTRACT

Pituitary LH release has been known to be regulated by the hypothalamic gonadotropin releasing hormone (GnRH) and the gonadal steroid hormones. In addition, neurotransmitters and neuropeptides are actively involved in the control of LH secretion. The alteration in LH release might reflect changes in biosynthesis and/or posttranslational processing of LH. However, little is known about the mechanism by which biosynthesis of LH subunits is regulated, especially at the level of transcription.

In order to investigate if ovarian steroid hormones regulate the LH subunit gene expression, α and LHβ steady state mRNA levels were determined in anterior pituitaries of ovariectomized rats. Serum LH concentrations and pituitary LH concentrations were increased markedly with time after ovariectomy. α and LHβ subunit mRNA levels after ovariectomy were increased in a parallel manner with serum LH concentrations and pituitary LH contents, the rise in LHβ subunit mRNA levels being more prominent than the rise in α subunit mRNA. α and LHβ subunit mRNA levels in ovariectomized rats were negatively regulated by the continuous treatment of ovarian steriod hormones for 1~4 days and LHβ subunit mRNA seemed to be more sensitive to negative feedback of estradiol than progesterone. Treatment of estrogen antagonist, LY117018 or progesterone antagonist, RU486 significantly restroed LH subunit mRNA levels as well as LH release which were suppressed by estradiol or progesterone treatment. These results suggest that ovarian steroids negatively regulate the LH synthesis at the pretranslational level by modulating the steady state levels of α and LHβ subunit mRNA and LHβ subunit mRNA seemed to be more sensitive to negative feedback action of estradiol than progesterone.

Key Words: α and LHβ subunit mRNA, Steroids, Pituitary, Rat

INTRODUCTION

Luteinizing hormone (LH) is a pituitary gonadotropin that plays an important role in the regulation of gametogenesis and gonadal steroidogenesis. LH, like follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), is a glycoprotein hormone and consists of α and β subunits, which are encoded by separate genes (Fiddes and Goodman, 1981). Glycoprotein hormones, within a species, consist of a nearly identical α subunit and a unique β subunit that confers the biological specificity to each hormone (Pierce and Parsons, 1981). The structures of α and LHβ subunit genes were determined (Jameson *et al.*, 1983) and cDNAs of rat α and LHβ subunits were synthesized (Chin *et al.*, 1983).

Pituitary release of LH is regulated by the hypothalamic GnRH and the gonadal steroid hormones. LH secretion follows two distinct and

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functionally separable modes, the preovulatory surge and the tonic release, and these are regulated by positive and negative feedback actions of ovarian steroids, respectively. In addition, neurotransmitters (Ramirez *et al.*, 1984) and neuropeptides (Panerai *et al.*, 1985) appears to be involved in the control of LH secretion.

The alteration in LH levels is presumed to reflect changes in biosynthesis and/or posttranslational processing. However, little is known about their regulation of the biosynthsis of LH subunits at transcriptional and posttranscriptional levels. During the last years, the availability of cDNA clones for α and LHβ subunit mRNAs allowed investigation of the regulation of subunit gene expression. The excess production of α subunit over LHβ subunit (Kourides *et al.*, 1980) has suggested that the rate of LHβ subunit synthesis determines the amount of intact LH produced (Gharib *et al.*, 1986).

To demonstrate the role of ovarian steroids in LH subunit synthesis at the level of transcription, we first studied the effect of ovarian steroid deprivation and ovarian steroid replacement on steady state levels of α and LHβ subunit mRNAs in anterior pituitaries of female rats.

MATERIALS AND METHODS

Experimental animal

Adult female Sprague Dawley rats weighing 180 ~200 g were used in the experiment. Animals were housed under controlled conditions (lights 07:00 h~21:00 h) with food and water freely available. In the first group, animals were sacrificed by decapitation on 1, 7, 14, 21, and 28 days after ovariectomy (OVX). In the second group, OVX rats (3 wks postcastration) were daily injected sc with vehicle (sesame oil), 17β-estradiol (20 ug/0.2 ml in sesame oil; Sigma Chem. Corp., U. S.A.) and/or progesterone (2 mg/0.2 ml in sesame oil; Sigma Chem. Corp., U.S.A.) for 1, 2 or 4 days and decapitated 24 hrs thereafter. In third group, OVX rats (3 wks postcastraction) were injected sc with estradiol and estrogen antagonist, LY117018 (20 ug/0.2 ml in sesame oil; Eli Lilly Corp., U.S.A.) or progesterone and progesterone antagonist, RU486 (2 mg/0.2 ml in sesame oil; Russel-UCLAF, France) and decapitated 24 hrs after.

Sera from trunk blood were stored at −20℃ for LH determination and anterior pituitaries for LH content and subunit mRNA levels were quickly frozen and stored in liquid nitrogen.

Radioimmunoassay (RIA) for rat LH (rLH)

Concentrations of serum and pituitary LH 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.) and LH levels were expressed in terms of rLH-RP-3 standards. rLH-I-4 was iodinated by chloramine-T method and ^{125}-I labeled LH (20,000~25,000 cpm/tube) was used. 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 12 %. Pituitary LH content was measured by RIA from supernatants of tissue homogenates.

Determinations 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. And γ-actin cDNA probe was used for internal standard. γ-actin cDNA was isolated from Okayama-Berg cDNA cloning expression vector with BamHI digestion. Each cDNA probes were labelled using random primer labelling kit (Amersham, UK) with [α-^{32}P]dCTP.

RNA preparation: Total RNA was isolated from individual rat pituitaries by nonidet P-40 method (Papavasiliou *et al.*, 1986). Briefly, pituitaries were homogenized by glass homogenizer containing 220 ul homogenization buffer (10 mM Tris-HCl, 1 mM EDTA, and 0.5 % (V / V) nonidet P-40, pH 7.5). The homogenates were centrifuged at 13,000 ×g at 4℃ for 30 min. The supernatant was transfered to a sterile tube and added proteinase K. After incubation (37℃, 30 min), sample was extracted with phenol/choroform. RNA was precipitated overnight at −20℃ with 3 M sodium acetate (0.1 Vol) and absolute ethanol (2.5~3 vol.). Total RNA was then recovered by centrifugation at 13,000 ×g at 4℃ for 30 min and resolved in distilled water.

Slot-blot and Northern-blot hybridization: In slot-blot hybridization, total RNA aliquots (2~

3 ug of total RNA from each sample) adjusted to 50 ul with sterile water were trasferred to sterile tube containing 30 ul of 20× SSC plus 20 ul of 37 % formaldehyde. The mixture was then incubated at 65℃ for 10 min, and chilled on ice. RNA was blotted to nitrocellulose membrane (0.45 um pore size) using slot blot apparatus (Minifold, Schleicher & Schuell, W. Germany). Wells were rinsed with 150 ul of 15×SSC and then baked in vacuum oven at 80℃ for 2 hours. In northern-blot hybridization, RNA was dissolved in D.W. and denatured in 50% formamide, 6.2% formaldhyde, 20 mM MOPS, 5 mM sodium acetate and 1 mM EDTA at 60℃ for 5 min. RNA was fractionated by size using electrophoresis on 1.2% agarose gel containing 6.2% formaldehyde and 20 mM MOPS. The RNA ladder was also denatured and electrophoresed on the gel for size markers. RNA was transferred onto nitrocellulose filters (0.45 um) according to the capillary blotting. After 3 hr transfer, the filters were dried in air and baked for 2 hr at 80℃.

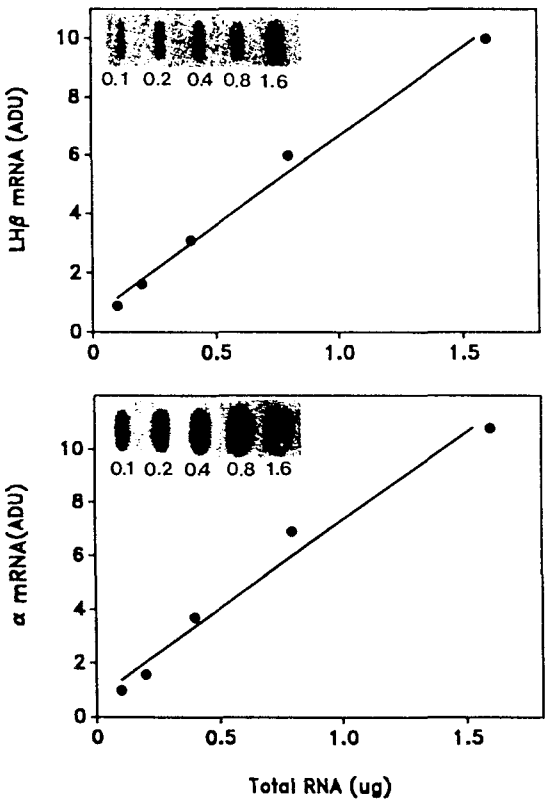

Fig. 1. Linear increase in α and LHβ subunit mRNA as a function of the amounts of total RNA blotted onto nitrocellulose membrane.

Hybridization and Autoradiography: The hybridization procedure was similar to that previously described by Papavasiliou *et al.*, (1982). Nitrocellulose filters were prehybridized in 50% deionized formamide, 5×SSC, 5× Denhardt's solution, 0.1% SDS and 2 mg heat-denatured salmon sperm DNA at 42℃ for 3 hr. After prehydridization, the blots were hybridized with ^{32}P-labelled probe at 42℃ for 18 hours. The blots were washed three times in 2×SSC and 0.1% SDS at room temperature and once in 0.1×SSC and 0.1% SDS at 50℃. The filters were then dried and exposed to X-ray film (Kodak X-Omat) at −70℃ for 4 hrs. Slot intensity in autoradiogram were assessed by scanning densitometer analysis. Dose-response curves with RNA dilutions have been determined and found to linear (Fig. 1). The integrated peak area of each slot was expressed as arbitrary densitometric unit (ADU) over intact group.

Statistical analysis

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

RESULT

Changes in serum LH concentrations and LHβ subunit mRNA levels in anterior pituitary gland after ovariectomy

As shown in Fig. 2A, serum LH concentrations were markedly increased with time after ovariectomy (OVX) in rats. Mean serum LH levels were increased from 1.02±0.13 ng/ml in intact rat to 6.00±0.52 ng/ml ($P<0.01$) by 14 days postcastration and to 6.82±1.54 ng/ml ($P<0.01$) by 28 day postcastration. Pituitary LH content was increased from 5.12±0.91 ng/mg protein to 18.5±1.35 ng/mg protein by 28 day postcastration. Steady state LHβ subunit mRNA levels in anteri-

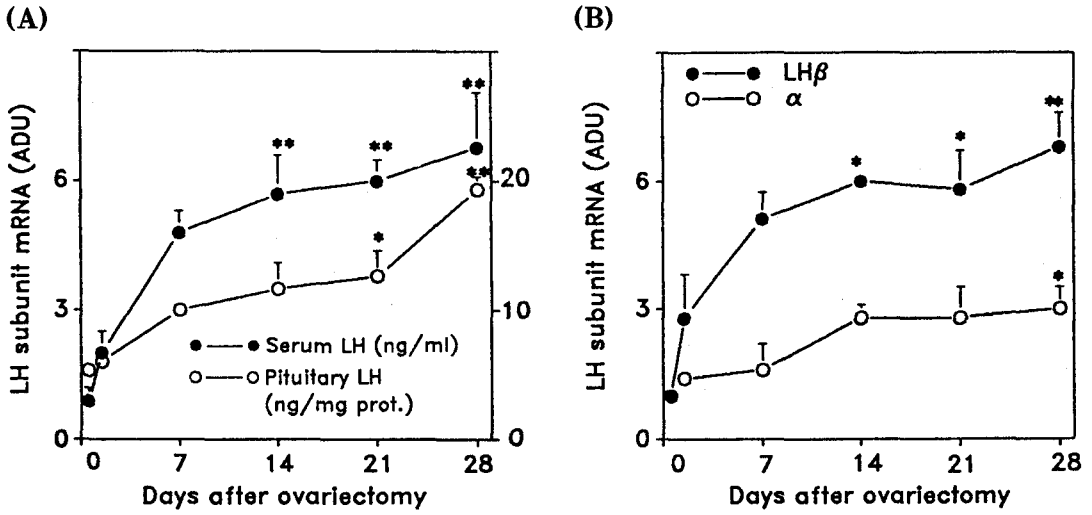

Fig. 2. Time courses of changes in (A) serum and pituitary LH concentrations and (B) α subunit and LHβ subunit mRNA levels after ovariectomy in rats. Each point represents the mean±S.E. of 6-7 animals. *$p<0.05$; **$p<0.01$ compared with control group.

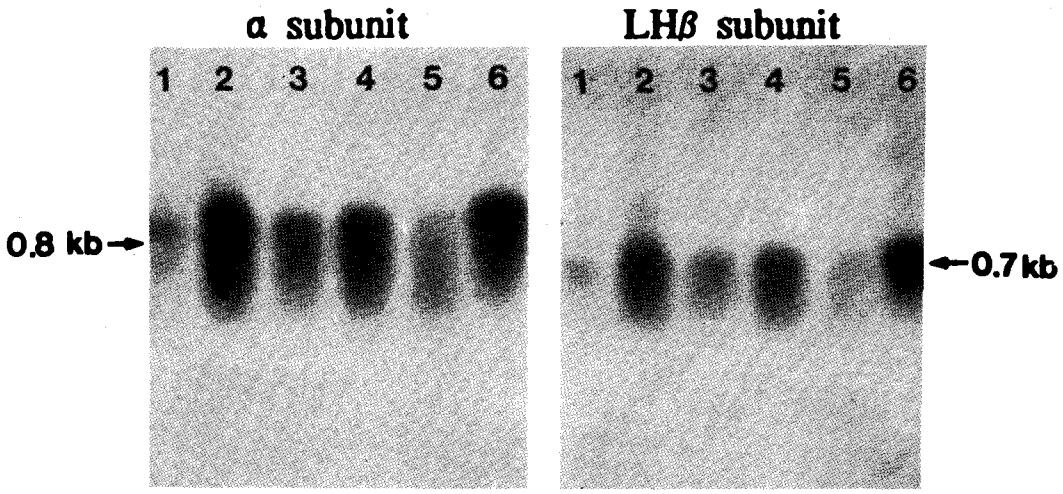

Fig. 3. Northern blot analysis of pituitry total RNA hybridized with P^{32}-labelled α and LHβ subunit cDNA probes. The position of the α (0.8 kb) and LHβ (0.7 kb) mRNA are indicated by arrows. lane 1, intact; lane 2, ovariectomized (OVX); lane 3; OVX+Estradiol (E; 80 ug/4 days); lane 4, OVX+Progesterone (P; 8 mg/4 days); lane 5, OVX+E (80 ug/4 days)+P(80 mg/4 days); lane 6, OVX+E(80 ug/4 days)+LY117018(80 ug/4 days).

or pituitary were increased in a parallel manner with serum LH concentrations after ovariectomy (Fig. 2B). By 14 days and 28 days postcastration, 6.0 fold (6.0±0.09 ADU) and 6.3 fold increases in LHβ subunit mRNA over intact control were observed, respectively ($P<0.01$). α subunit mRNA

level was also increased gradually, reaching the maximum (3.0±0.47 ADU) on 28 days postcastration ($P<0.05$), the rise in LHβ subunit mRNA levels being more prominent than the rise in α subunit mRNA.

Northern blot analysis of electrophoretically separated cellular RNA hydridized with specific probes of α and LHβ subunit cDNAs is shown in Fig. 3. Since after hybridization with LHβ subunit cDNA probe, this probe was washed, and α subunit probe rehybridized with the washed NC paper, the intensity of the band on the autoradiograms can be used as a measure of the ralative amount of specific mRNA present in the pituitary gland. As shown in Fig. 3, relative amount of α subunit mRNA exceeded LHβ subunit mRNA.

Effects of ovarian steroid replacement on LH release and subunit mRNA levels in anterior pituitary glands of ovariectomized rats

The treatment of estradiol or progesterone lowered serum LH concentrations induced by ovariectomy (Fig. 4). Estradiol (100 ug/kg body weight) or progesterone (P: 10 mg/kg body weight) were daily administrated to ovariectomized rats starting treatment (21 days postcastration) for 1, 2, or 4 days and sacrificed at 24 hours after last treatment. Administration of estradiol for 1 day singnificantly declined LH release to 2.45±0.75 ng /ml by about 50% ($P<0.01$). After the treatment of estradiol of 4 days, serum LH concentration was decreased to 1.15±0.93 ng/ml, which was similar to serum LH level of non-ovariectomized rats. Administration of progesterone did not show significantly suppressive effects on serum LH concentration (Fig. 4). The treatment of progesterone for 1 and 2 days did not significantly decreased serum LH concentration but 4 days treatment significantly decreased serum LH concentration to 3.85±1.02 ng/ml. Concomitant treatment of progesterone with estradiol also significantly decreased serum LH concentration.

As shown in Fig. 5, LHβ subunit mRNA levels were not significantly decreased by the treatment of estradiol for 1 day, but they were significantly declined to 3.89±0.35 ADU ($P<0.05$) or 1.65±0.78 ADU ($P<0.01$) by 2 days or 4 days treatment, respectively. α subunit mRNA levels were tended to decline by 1 day or 2 days treatment but they

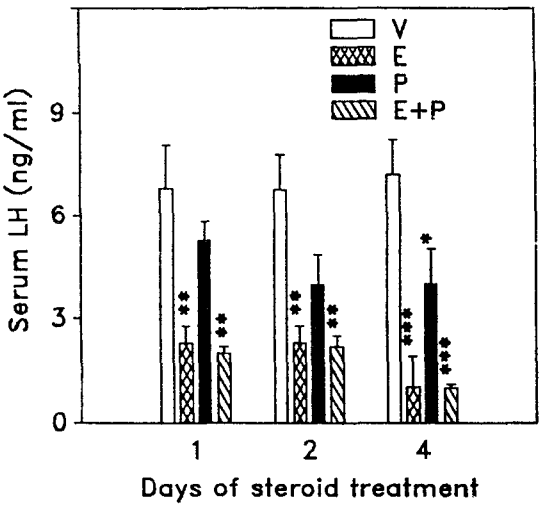

Fig. 4. Effects of ovarian steroid hormones (estradiol, 20 ug/progesterone, 2 mg) treated for different durations on serum LH concentrations of ovariectomized rats. Each bar represents the mean±S.E. of 6 animals. Ovariectomy (OVX); Vehicle (V); Estradiol (E); Progesterone (P)
*p<0.05, **p<0.01, ***p<0.001; compared with vehicle-treated group.

were not statistically significant. With treatment of estradiol for 4 days, α subunit mRNA levels were signficiantly decreased to 1.35±0.58 ADU ($p<0.05$).

Administration of progesterone did not show significant suppressive effects on LH Subunit mRNA levels (Fig. 5). Treatment of progesterone did not alter the α and LHβ subunit mRNA level, compared with vehicle treated group but tended to decrease α and LHβ subunit mRNA levels. Moreover, concomitant treatment of progesterone with estradiol significantly decreased α and LH subunit mRNA levels.

Effects of antagonists for ovarisn steroids on LH release and LH subunit mRNA levels

In order to confirm the inhibitory effects of ovarian steroid hormones on the LH release and LH subunit mRNA levels, ovariectomized rats (21 days after postcastration) were treated with estradiol and estrogen antagonist, LY117018, or progesterone and progesterone antagonist, RU486, for 4

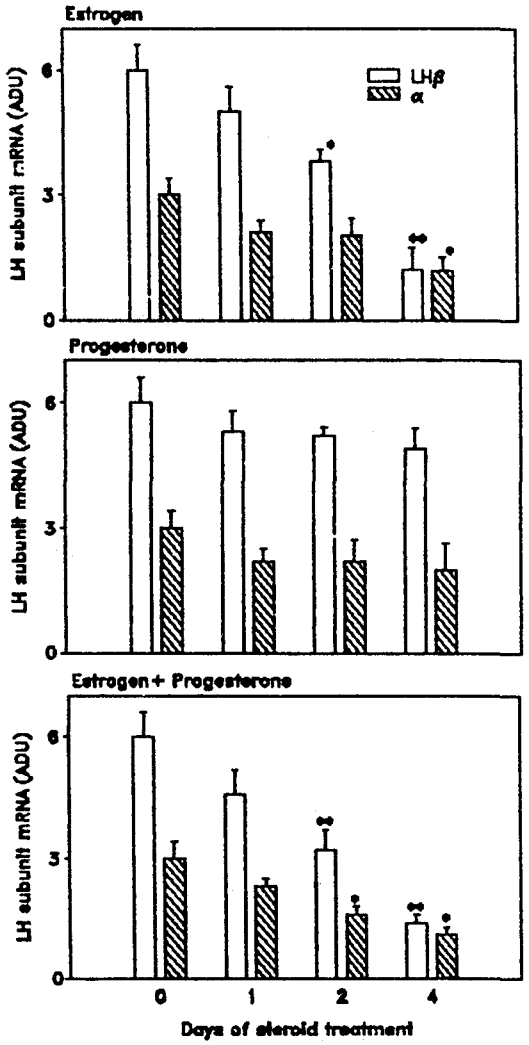

Fig. 5. Effects of ovarian steroid hormones (estradiol, 20 ug; progesterone, 2 mg) treated for different duration on α subunit and LHβ subunit mRNA levels in anterior pituitary gland of ovariectomized rat. Each bar represents the mean±S.E. of 6 animals.
*p<0.05, **p<0.01; compared with control group.

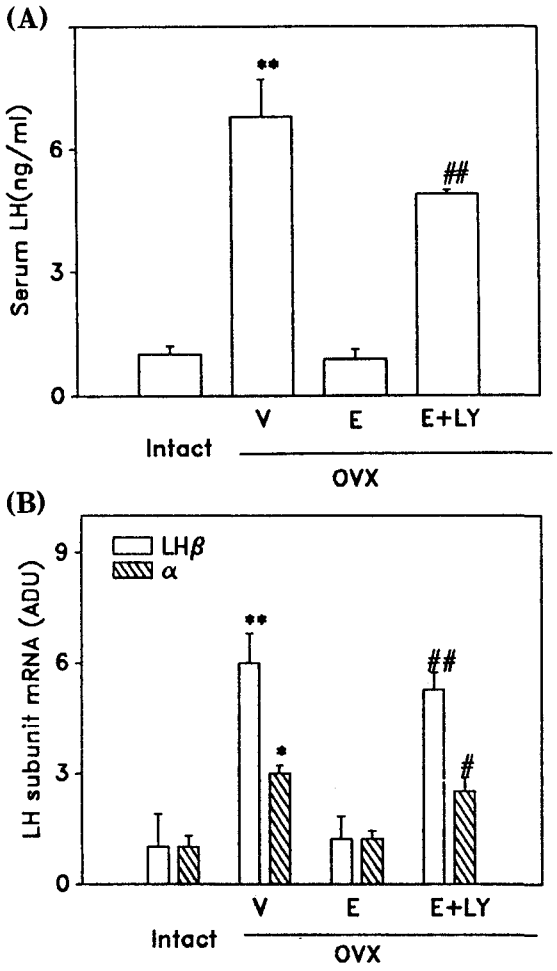

Fig. 6. Effects of estrogen antagonist LY117018) on estradiol induced decrease in (A) serum LH concentrations and (B) α subunit and LHβ mRNA levels of ovarectomized rat. Each bar represent the mean±S.E. of 5 animals. V, Ovariectomized (OVX) control; E, Estradiol; E+LY, Estradiol+LY117018
*p<0.05, **p<0.01; compared with OVX+V group.
#p<0.05, ##p<0.01; compared with OVX+E group.

days. As showm in Fig. 6, estradiol-induced decreases in α and LHβ subunit mRNA levels as well as LH release were significantly recovered by the treatment of LY117018. Serum LH concentrations were increased from to 5.02±0.16 ng/ml (p<0.01) by the concomitant treatment of estradiol and LY117018 for 4 days. Also α and LHβ subunit mRNA levels were restored to 2.62±0.51 ADU

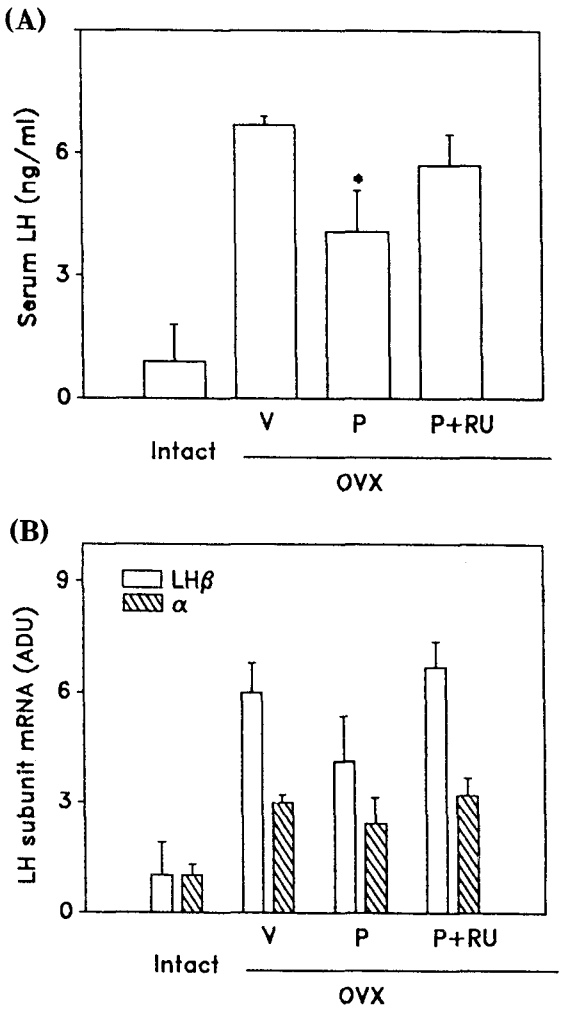

Fig. 7. Effects of progesterone antagonist (RU486) on progesterone induced decrease in (A) serum LH concentrations and (B) α subunit and LHβ mRNA levels of ovariectomized rat. Each bar represent the mean±S.E. of 5 animals. V, Ovariectomized (OVX) control; P, Progesterone; P+RU, Progesterone+RU486
*$p<0.05$; compared with OVX+V group

and 5.35±0.46 ADU.

Concomitant treatment of progesterone and RU486 also tended to recover LH release and subunit mRNA levels up to the levels of ovariectomized control, although the differences were statistically insignificant.

DISCUSSION

In these studies, we examined the time course and magnitude of changes in LH subunit mRNA concentrations after castration and correlate α subunit mRNA levels with serum and pituitary LH levels. Ovariectomized rats offer an excellent system for studying LH subunit gene transcription, bacause of high levels of LH production as resulted by the removal of negative feedback of ovarian steroids.

It has been well known that castration increases serum concentrations (Wheaton and McCann, 1976; Wise and Ratner, 1980; Buterstein *et al.*, 1983) and pituitary content of gonadotropins (Yamamoto *et al.*, 1970) which indirectly suggest castration increases gonadotropin synthesis. In male rats, the majority of increase is seen 24 h post orchidectomy, whereas in female rats, the increase in gonadotropins occurs more slowly, Serum LH concentrations were rapidly increased reaching the peak value of four to 20- fold higher than control values at 2-3 weeks postcastration (Badger *et al.*, 1978; Corbani *et al.*, 1984). In our study, serum LH levels were 6.8 fold increased above control levels by 28 days after ovariectomy. Gonadectomy has also been reported to lead to prompt increases in LH subunit mRNA levels in male and female rats. However, the magnitude and time-course of the postcastration rise in serum LH concentrations and subunit mRNA appeared to vary. Several studies reported that four − to 15− fold increase in pituitary levels of both α and LHβ subunit mRNAs after ovariectomy (Corbani *et al.*, 1984; Abbott *et al.*, 1985; Papavasiliou *et al.*, 1986). Our results are consistent with these data. As shown in Fig. 2B, α and LHβ subunit mRNAs were gradually increased, reaching the maximum mRNA responses at 3~4 weeks postcastration. α and LHβ subunit mRNA were 6.3 fold and 3.0 fold increased compared with those of intact rat by 28 days postcastration. Our results are also supported by in situ hydridization studies that the number of LHβ mRNA containing cell as well as amount of LHβ mRNA per cell were increased by gonadectomy (Childs *et al.*, 1987; Kotsuji *et al.*, 1992).

The exact role of LH subunit mRNAs in the synthesis of LH is uncertain, but the excess of α subunit has led to suggestion that availability of β subunit is the rate-limiting step (Godine *et al.*, 1980; Fetherstone, 1982; Papavasiliou *et al.*, 1986). Examination of the mRNA responses to castration provides some support for this suggestion. An excess of α mRNA over LHβ mRNA was observed in intact rats by northern blot hybridization method. In cell-free translation studies (Fetherston and Boime, 1982; and Corbani *et al.*, 1984) and slot blot hybridization studies (Gurr and Kourides, 1984; Gharib *et al.*, 1986), α subunit mRNA levels exceed LHβ and TSHβ subunit mRNA levels in various physiological situations. Moreover, it is known that α subunit biosynthesis is also regulated by thyroid hormones (Croyle and Maurer, 1984; Chin *et al.*, 1985) as well as gonadal steroid hormones (Couis *et al.*, 1983; Landefeld *et al.*, 1984). Leung *et al.*, (1988) reported that steay state mRNA levels and transcription rate of α subunit gene did not vary significantly throughout 4 day estrous cycle, but there are distinct and divergent alterations for LHβ and FSHβ. Therefore these findings support that synthesis of the LHβ subunit is the rate limiting step in the biosynthesis of LH.

It has long been established that gonadal steroid hormones negatively and positiviely regulate gonadotropin secretion. Treatment of castrated rats with gonadal steroid hormone lowers plasma LH levels (Rabii and Ganong, 1976; Schanbacher and Ford. 1977), which are consistent with the present results. However, little evidence has been accumulated that gonadal steroid hormones regulates gene expression of gonadotropin subunits. In this study, we examined the effects of steroid hormones on LH subunit synthesis at the pretranslational level in ovariectomized rats. Replacement of estrogen to ovariectomized rat restored intact levels of α, and LHβ subunit mRNA as well as LH secretion. The daily treatment of ovariectomized rat with estradiol (20 ug/day) lowerd serum LH levels and LH subunit mRNA levels. Furthermore, the treatment of estrogen antagonist (LY117018) significantly recovered LHβ subunit mRNA level as well as LH release which were decreased by the treatment of estradiol. Cell-free translation studies have shown that both α (Godine *et al.*, 1980) and LHβ subunit mRNA (Counis *et al.*, 1983) levels were lower in estradiol treated ovariectomized ewes than untreated control (Landefeld *et al.*, 1983). Using blot hybridization analysis, others have also shown that treatment of ovariectomized ewes and rats with estradiol results in a dramatic reduction in steady state α subunit and LHβ subunit mRNA levels (Nilson *et al.*, 1983). Our data together with others indicate that estrogen may play an integral role in the regulation of LHβ subunit gene expression as well as LH secretion.

Whether progesterone is involved in the regulation of gonadotropin subunit synthesis has been controversial. The results of an investigation by Counis *et al.* (1983) showed, using cell free translation techniques, that progesterone had no effect on gonadotropin subunit mRNA levels in ovariectomized rat. On the other hand, many studies showed that role of progesterone appeared to depend on the prevailing gonadal steroid milieu. Simard *et al.* (1988) have found that progesterone alone exerts no effect on α and LHβ subunit mRNA levels in castrated rats whereas if it was administrated with estradiol, suppression of LH subunit mRNAs as observed in both male and female rats. But our data showed that progesterone only decreased serum LH levels. LH subunit mRNA levels tended to be decreased by daily treatment of progesterone alone, although decrease was not statistically significant. The concomitent treatment of progesterone antagonist (RU486) with estrogen restored serum LH levels and LHβ subunit mRNA level. These results agree with Dalkin's report (1990). Dalkin *et al* reported that progesterone replacement to 7 day ovariectomized rat did not alter α, LHβ or FSHβ mRNAs. Additionally, progesterone given at the time of ovariectomy did not prevent the rise in gonadotropin subunit mRNAs, and progesterone implant for up to 7 days suppressed α and LHβ subunit mRNAs as well as pituitary LH contents. It is, therefore, suggested that α and LHβ subunit mRNA levels are negatively regulated by the continuous treatment of ovarian steroid hormones for 1~4 days after ovariectomy and LHβ subunit mRNA seemd to be more sensitive to negative feedback by estradiol than progesterone.

In the present study, we measured steady state LHβ mRNA levels to evaluate gene expression of LHβ subunit. However, changes in steady state mRNA levels can be due to alterations in tran-

scription rate, or to change in the rate of mRNA turnover or stability. By using a nuclear run-off assay, Shupnik *et al* (1988) have found that the synthesis of gonadotropin subunit mRNAs from ovariectomized rats was significantly greater than that from intact rats (α, 2.5 fold; LHβ, 10 fold). In addition, estradiol treatment of ovariectomized rats resulted in marked declines in the synthesis of LH subunit mRNAs (α, 70%; LHβ, 88%). The magnitude of these changes correlates well with the changes in steady state LH subunit mRNA levels that we found in this studies. This resuls suggests that steady state mRNA changes of LHβ subunits can be accounted for, in part, by changes in transcripton of the LHβ genes.

It is not possible from our experiment to ascertain if estradiol acts directly on the gonadotrope cells or indirectly via the hypothalamus to regulate GnRH activity, or by a combination of both pathways. Estradiol could act directly on ovine pituitary cell to suppress α and FSHβ mRNA (Hall and Miller, 1986; Gharib *et al.*, 1987; Phillips *et al.*, 1988), and decrease the number of GnRH receptors on gonadotropes in rat thereby modulating the pituitary responsiveness to GnRH (Miller and Wu, 1981; Frawley and Neil, 1984). Moreover, discrepant changes in GnRH and gonadotropin secretion further suggest that pituitary is an important site of negative feedback control (Kitahara *et al.*, 1990; Dalkin *et al.*, 1992). On the other hand, GnRH may be an important factor for the regulation of LH synthesis as well as LH release since GnRH secretion is increased after castration (Frager *et al.*, 1981). However, estradiol treatment of hypothalamic tissue slice decreased hypothalamic GnRH mRNA (Wray *et al.*, 1989). Thus, both direct effect on the gonadotropes by alterating the response to GnRH, and indirect effect via hypothalamic secretion of GnRH could be responsible for the regulation of LH subunit gene expression by estradiol.

In our laboratory, further studies to elucidate the mechanism by which ovarian steroids regulate gene expression of LHβ subunit at the pituitary level have been undertaken in in vitro pituitary culture system.

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=국문초록=

난소제거된 흰쥐에서 난소호르몬에 의한 LHβ subunit의 유전자 발현조절

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난소호르몬에 의하여 황체형성호르몬(luteinizing hormone; LH) subunit의 유전자 발현이 어떻게 조절되는가를 조사하기 위하여 성숙한 흰쥐에서 난소를 제거하거나 또한 난소호르몬을 재투여한 후 α 및 LHβ subunit mRNA의 수준을 조사하여 다음과 같은 결과를 얻었다.

1. 난소를 제거한 후 시간이 경과함에 따라 혈중 LH 농도 및 뇌하수체 LH 함량이 급격히 증가하였다. 또한 난소제거 후 14일 후부터 α subunit mRNA 수준이 증가하기 시작하였으며, LH β subunit mRNA 수준은 난소제거 후 1일부터 증가하기 시작하여 혈중 LH 농도와 같은 양상으로 증가하였다.

2. 난소제거 후 21일 경과후에 난소호르몬을 투여하였을때 난소제거로 증가된 혈중 LH 농도와 α 및 LHβ subunit mRNA 수준이 감소하였다. Estradiol을 1일간 투여하였을때 부터 혈중 LH 농도 및 α와 LHβ subunit mRNA 수준이 감소하였으며, progesterone을 4일간 처리하였을때에 혈중 LH농도가 감소하였다.

3. Estrogen 길항제인 LY117018를 estradiol과 동시에 처리하거나, progesterone 길항제인 RU 486을 progesterone과 동시에 처리하였을때 estradiol과 porgesterone에 의하여 감소되었던 혈중 LH 농도 및 α와 LHβ subunit mRNA 수준이 유의하게 회복되었다.

이상의 결과로 보아 LH 분비에 있어서 LHβ subunit mRNA 수준의 변화가 속도결정단계(rate limiting step)인 것으로 보이며, 난소홀몬은 α 및 LHβ subunit mRNA 수준을 조절하므로써 pretranslation 단계에서 LH 생합성을 조절하는 것으로 생각된다.