

Sex Steroid Hormone Regulation of Follicle-stimulating Hormone Subunit Messenger Ribonucleic Acid (mRNA) Levels in the Rat

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Abstract

Follicle-stimulating hormone (FSH) β , luteinizing hormone (LH) β , and α subunit messenger RNA (mRNA) levels were examined in rats after castration and sex-steroid replacement. Subunit mRNAs were determined by blot hybridization using rat FSH β genomic DNA, and α and LH β complementary DNA (cDNA). Rat FSH β mRNA is 1.7 kilobase in size. After ovariectomy, female FSH β mRNA levels increased fourfold, whereas those of LH β and α increased twenty- and eightfold, respectively. With estradiol, all subunits returned toward normal levels. Male LH β and α mRNA levels rose eight- and fourfold, respectively, 40 d postcastration, but FSH β mRNA levels increased minimally. After 7 d of testosterone propionate, LH β and α mRNAs declined to normal levels, whereas FSH β mRNA increased slightly. We conclude that in female rats FSH β is negatively regulated by gonadal steroids, but to a lesser extent than LH β or α mRNAs, and there is a differential regulation of FSH β mRNA levels in males as compared with females at the time points examined.

Introduction

Follicle-stimulating hormone (FSH),¹ like luteinizing hormone (LH), is a glycoprotein hormone that is synthesized in the gonadotropes of the pituitary gland. It consists of two dissimilar, non-covalently bound subunits: an α subunit that, within a species, is identical in both hormones, and a unique β subunit that determines the biologic specificity of the hormone (1). FSH is critical in the control of gonadal function and is essential for gametogenesis (1).

Much is known about the regulation of secretion of FSH including its stimulation by LH-releasing hormone (LHRH) (2), and its inhibition by sex steroid hormones (3–5) and inhibin (6–9). Little is known, however, about the regulation of the biosynthesis of the subunits of FSH at the pretranslational level.

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1. *Abbreviations used in this paper:* ADU, arbitrary densitometric units; CAST, castrated; E₂, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone, LHRH, LH-releasing hormone; T, testosterone propionate.

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Using cell-free translation, an indirect method of quantitation of messenger RNA (mRNA) levels, investigators have shown that estradiol (E₂) suppresses FSH β mRNA levels in castrated (CAST) rats and in cultured female ovine pituitary cells (10). Also, others have reported that FSH β mRNA translational activity in female rat anterior pituitaries increased with ovariectomy and declined to undetectable levels with E₂ treatment (11). We and others have demonstrated the negative regulation of α and LH β subunit mRNAs by sex steroid hormones (12–15). Examination of FSH β subunit steady state mRNA levels has not been possible until recently, however, because of the lack of availability of a suitable FSH β DNA probe.

In this study, we have used a rat FSH β genomic DNA fragment probe to investigate the hormonal regulation of FSH β mRNA in two physiologic models: (a) CAST rats and (b) CAST rats treated with sex steroids. Our results indicate that in female rats FSH β mRNA levels are negatively regulated by gonadal steroids, but to a lesser extent than LH β or α subunit mRNAs. In male rats, however, there is a minimal regulation of FSH β by testosterone, in contrast to the moderate E₂-mediated negative regulation of FSH β mRNA levels observed in the female.

Methods

Experimental protocols. Adult male and female Sprague-Dawley rats (CD strain, 200–225 g; Charles River Breeding Laboratories Inc., Wilmington, MA) were used in all experiments. Two experimental models were used concurrently. In the first, the castration time course model, adult male and female rats were CAST and then killed at 0, 1, 7, 14, 21, and 28 d postcastration. In the second, the sex steroid-replacement model, adult male and female rats 40 d postcastration received daily subcutaneous injections with sex steroid hormones. Males were injected with testosterone propionate (T) (500 μ g/100 g body wt) and females were injected with 17 β -E₂ benzoate (10 μ g/100 g body wt). Animals were then killed 0, 1, and 7 d after injections were initiated. In both models 16–18 animals were killed for each time point. 12 animals were used for subunit mRNA determinations, and 4–6, for measurement of pituitary FSH and LH concentrations. All animals were killed by decapitation and trunk blood was collected for serum FSH and LH determinations. Whole pituitaries were carefully dissected, quick-frozen, and stored in liquid nitrogen.

Radioimmunoassay (RIA) of FSH and LH. Serum levels of rat FSH and LH were determined by RIAs using reagents from the National Institute of Arthritis, Metabolism, and Digestive Diseases (Bethesda, MD) as described previously (16), except that RP-2 standards were used. Pituitary concentrations of FSH and LH were also determined by RIA in the same fashion using extracts prepared from pituitaries homogenized in phosphate-buffered saline (16).

DNA Probes. Synthetic rat α and LH β subunit complementary (cDNAs), and mouse β -actin cDNA have been described previously (14). Also, a 1.0-kilobase (kb) genomic DNA fragment encoding a portion of the rat FSH β subunit gene was isolated from a rat Eco RI genomic library by using a bovine FSH β cDNA probe (generously provided by E. Bernstine, Integrated Genetics Inc., Framingham, MA). DNA sequence

analysis shows that the fragment contains ~ 600 basepair (bp) of the third exon of the rat FSH β gene of which 225 bp encode the last 75 amino acids of carboxy-terminal end of the subunit and the remaining bp, the 3'-untranslated region (unpublished data). DNA fragments were labeled using random-primer translation (17) to achieve a specific activity of 0.5×10^9 – 1.0×10^9 cpm/ μ g DNA.

Subunit mRNA determinations. 12 pituitaries were used for each time point. Total RNA was extracted from pools of two pituitaries as described previously (14) so that there were six RNA samples for each time point. Two sets of Northern blots were made as follows. For the first set, 5 μ g of total RNA (OD₂₆₀) from each sample were denatured with formaldehyde and subjected to electrophoresis on a 1.4% (wt/vol) agarose gel (in 2 mM 3-[N-morpholino]propane sulfonic acid, 500 μ M sodium acetate, pH 7.0, 100 μ M EDTA, 0.12 M formaldehyde, and 0.08 mg/dl [wt/vol] ethidium bromide). The RNAs were then transferred to nitrocellulose paper by diffusion blotting (18). The second set of blots were identical to the first, except that 15 μ g of total RNA from each sample were used. The blots were baked at 80°C for 2 h before hybridization.

The 5- μ g blots were hybridized successively to 5'-end-labeled α and LH β synthetic oligonucleotide probes using conditions described previously (14). The radiolabeled FSH β DNA fragment was hybridized to the 15- μ g blots using a hybridization buffer consisting of a 40% (vol/vol) formamide, 4 \times SSC (1 \times SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7), 7 mM Tris-HCl, 1 \times Denhardt's solution (0.02% [wt/vol] Ficoll-400, 0.02% [wt/vol] bovine serum albumin, and 0.02% [wt/vol] polyvinyl pyrrolidone-40), 2 μ g/ml sonicated-denatured salmon sperm DNA, and 10% (wt/vol) dextran sulfate. Hybridization was allowed to occur overnight (16–20 h) at 42°C. Finally, both sets of blots were hybridized with the labeled β -actin fragment (14).

After hybridization with the FSH β probe, blots were washed in 2 \times SSC/0.1% sodium dodecyl sulfate at 50°C. The washing conditions used after hybridizations with the α , LH β , and β -actin probes have been described (14). The blots were then subjected to autoradiography at –70°C with an intensifying screen. Blots hybridized with the FSH β probe were autoradiographed using two intensifying screens at –70°C. Band densities were determined by semiquantitative analysis with scanning densitometry. Dose–response curves with RNA dilutions have been determined previously (14) and were found to be linear.

Standardization of data. The total amounts of RNA in each lane of any given blot were internally standardized by determining the level of β -actin mRNA for each sample and correcting the α , LH β , and FSH β subunit mRNA levels accordingly (14).

Statistical analysis. *t* test for independent samples was used to analyze the data from the determinations for pituitary concentrations of FSH and LH, and a Wilcoxon rank-sum test (Mann–Whitney variation) was used for all other data.

Results

Females

Castration time course. The time course of the changes postcastration in serum and pituitary gonadotropins as well as subunit mRNA levels were examined in female rats. With castration, serum FSH increased fivefold ($P < 0.001$) by 3 d or sixfold ($P < 0.01$) by 7 d postcastration and remained at these levels at the 21- and 28-d time points (Table I). In contrast, the earliest significant rise, a ninefold elevation ($P < 0.001$), in serum LH was a 7 d postcastration, and levels were still increasing at 28 d postcastration, when values were 23-fold elevated ($P < 0.001$), as compared with the levels observed at 0 d.

Pituitary concentrations of FSH and LH also increased gradually after castration in female rats, although the magnitude of these changes were much less dramatic than those seen in serum. Pituitary FSH concentration increased fourfold ($P < 0.05$) by 3 d and fivefold ($P < 0.05$) by 21 d postcastration (Table I).

Table I. Time Course of Changes in Serum and Pituitary Concentrations of FSH and LH Postcastration in Female and Male Rats

| | Serum | | Pituitary | |
|---------------|-------------------------------|-----------------------------|----------------------------|-----------------------------|
| | FSH | LH | FSH | LH |
| | ng/ml | ng/ml | μ g/mg protein | μ g/mg protein |
| Female | | | | |
| Intact | 5.5 \pm 1.5 | 0.3 \pm 0.2 | 0.3 \pm 0.1 | 3.3 \pm 1.0 |
| 3 d | 26.4 \pm 5.9* | 1.1 \pm 0.9 [‡] | 1.3 \pm 0.4 [§] | 4.3 \pm 1.2 [‡] |
| 7 d | 34.4 \pm 10.5 | 3.1 \pm 1.1 | 1.3 \pm 0.3 [§] | 7.0 \pm 2.2 [§] |
| 14 d | 38.0 \pm 13.8 [†] | 5.3 \pm 1.8 | 1.4 \pm 0.2 [§] | 7.9 \pm 0.7 [§] |
| 21 d | 35.2 \pm 9.4 | 6.1 \pm 1.4 | 1.6 \pm 0.3 [§] | 8.4 \pm 1.3 [§] |
| 28 d | 40.6 \pm 10.4 | 7.6 \pm 2.0 | | |
| Male | | | | |
| Intact | 10.4 \pm 1.8 | 0.4 \pm 0.1 | 4.0 \pm 1.6 | 6.8 \pm 1.1 |
| 3 d | 26.2 \pm 4.8* | 3.4 \pm 0.9 | 2.4 \pm 0.7 [‡] | 6.5 \pm 1.4 [‡] |
| 7 d | 33.1 \pm 7.8 | 3.8 \pm 1.4 [†] | 2.1 \pm 0.3 [§] | 7.5 \pm 1.5 [‡] |
| 14 d | 34.8 \pm 9.3 | 3.9 \pm 1.2 [†] | 3.1 \pm 1.0 [‡] | 15.7 \pm 5.6 [§] |
| 21 d | 35.3 \pm 6.0 | 4.8 \pm 1.0 [†] | 2.9 \pm 0.3 [‡] | 14.5 \pm 1.5 [§] |
| 28 d | 35.7 \pm 7.5 | 4.1 \pm 1.2 [†] | | |

All data expressed as mean \pm SEM. All data points are compared with intact levels.

* $P < 0.0005$.

[‡] $P = \text{NS}$.

[§] $P < 0.05$.

^{||} $P < 0.001$.

[†] $P < 0.005$.

Pituitary LH concentrations increased twofold ($P < 0.05$) at 7 d postcastration and remained elevated for the rest of the time course.

Sex steroid replacement. Serum and pituitary FSH and LH concentrations and subunit mRNA levels were determined in normal, CAST (40 d postsurgery), and CAST female rats that were replaced with E₂ for 7 d. Both serum FSH and LH levels increased markedly with ovariectomy when compared with intact animals. In the CAST females, serum FSH and LH were higher than the levels observed in normal female rats. With E₂ replacement, both FSH and LH levels declined markedly by 7 d, approaching the levels in intact females (Table II). Serum LH declined to a greater degree than did FSH. Pituitary FSH and LH concentrations increased seven- and threefold, respectively, by castration. They declined only minimally, however, with 7 d of E₂ treatment, and the decreases were not statistically significant.

FSH and LH subunit mRNA concentrations were determined by RNA blot hybridization and semiquantitative scanning densitometry of autoradiographic X-ray bands. A representative autoradiogram is shown in Fig. 1. The rat FSH β mRNA was found to be ~ 1.7 kb in size. With castration, FSH β mRNA levels increased only fourfold ($P < 0.001$), whereas LH β and α increased twenty- ($P < 0.05$) and eightfold ($P < 0.001$), respectively. With E₂ treatment, all three subunit levels decreased markedly, approaching normal levels (Fig. 2). There were early, statistically significant rises in all three subunit mRNAs (Fig. 3). Note, however, by 28 d postcastration FSH β subunit mRNA levels were fivefold elevated as compared with normals ($P < 0.001$), whereas those of LH β and α were thirty- ($P < 0.05$) and fourfold ($P < 0.001$) elevated, respectively.

Table II. Serum and Pituitary Concentrations of LH and FSH in Intact, CAST, and CAST Rats Treated with E₂ (Females) or T (Males) for 1 or 7 d

| | Serum | | Pituitary | |
|---------------|------------|----------|---------------|---------------|
| | FSH | LH | FSH | LH |
| | ng/ml | ng/ml | μg/mg protein | μg/mg protein |
| Female | | | | |
| Intact | 4.3±1.4 | 0.3±0.0 | 0.3±0.1 | 3.3±1.0 |
| Cast | 47.0±16.0* | 8.4±1.1* | 2.1±0.4* | 11.0±1.8* |
| 1 d | 35.4±4.9* | 2.8±1.0* | 2.3±0.0* | 11.7±0.6* |
| 7 d | 18.2±3.9* | 1.2±0.3* | 1.7±0.4‡ | 10.1±1.3‡ |
| Male | | | | |
| Intact | 11.2±1.1 | 0.6±0.2 | 4.0±1.6 | 6.8±1.1 |
| Cast | 37.5±5.4* | 6.9±1.8* | 3.5±0.9§ | 18.9±4.1 |
| 1 d | 35.3±3.5* | 2.2±1.7* | 4.0±0.9§ | 19.0±5.6 |
| 7 d | 26.0±2.3* | 0.2±0.1* | 4.4±0.5§ | 12.4±1.3 |

Data are expressed as mean±SEM. All data points are compared with intact levels.

**P* < 0.005.

‡*P* < 0.001.

§*P* = NS.

||*P* < 0.05.

Males

Castration time course. With castration, serum FSH and LH in male rats increased three- (*P* < 0.001) and ninefold (*P* < 0.001), respectively, by 3 d after orchietomy (Table I). By 7 d postcastration, both serum FSH and LH reached peak values, three- (*P* < 0.001) and tenfold (*P* < 0.05), respectively, above normal levels and remained elevated for the rest of the time course.

Pituitary LH concentrations also increased gradually with time postcastration. By 14 d postcastration pituitary LH concentrations had increased twofold (*P* < 0.05) as compared with intact levels (Table I). Pituitary FSH concentrations, in contrast, did not rise significantly above normal levels at any point after gonadectomy.

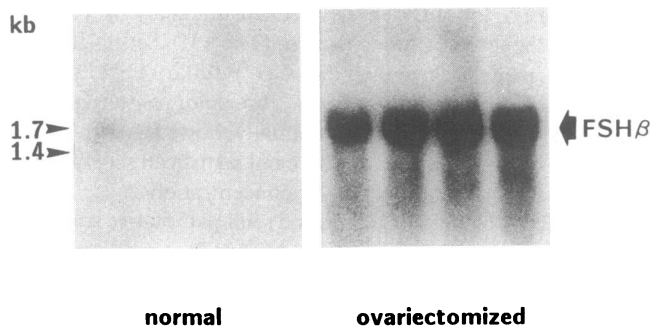


Figure 1. Rat FSH β mRNA: Northern blot hybridization analysis. A radiolabeled genomic fragment encoding the rat FSH β subunit was hybridized to total cellular RNA from the pituitary glands of intact and ovariectomized female rats as described in Methods. Each lane contains 15 μ g of RNA from a pool of two pituitary glands. The size markers to the left of the bands indicate the sizes of DNA fragments resulting from a digestion of pBR322 with Ava II. Film exposure time was 48 hours using Kodak XAR film and two intensifying screens at -70°C .

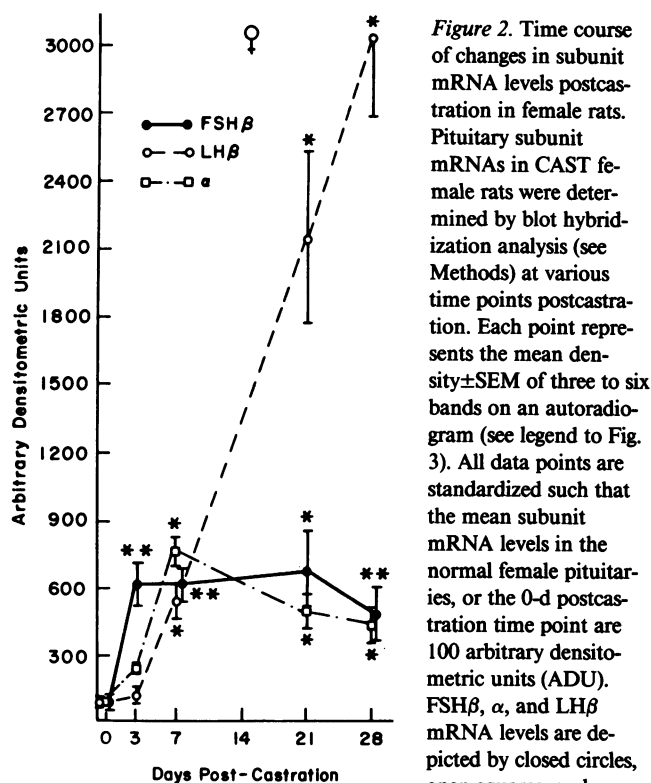


Figure 2. Time course of changes in subunit mRNA levels postcastration in female rats. Pituitary subunit mRNAs in CAST female rats were determined by blot hybridization analysis (see Methods) at various time points postcastration. Each point represents the mean density±SEM of three to six bands on an autoradiogram (see legend to Fig. 3). All data points are standardized such that the mean subunit mRNA levels in the normal female pituitaries, or the 0-d postcastration time point are 100 arbitrary densitometric units (ADU). FSH β , α , and LH β mRNA levels are depicted by closed circles, open squares, and open

circles, respectively. Stars represent statistical significance of data points as compared with the values for normal rats or 0 d postcastration: **P* < 0.05 and ***P* < 0.001.

Gonadotropin subunit mRNA levels paralleled the changes in pituitary concentrations of FSH and LH after orchietomy in male rats (Fig. 4). By 28 d postcastration, LH β and α subunit mRNA levels increased four- (*P* < 0.001) and twofold (*P* < 0.05),

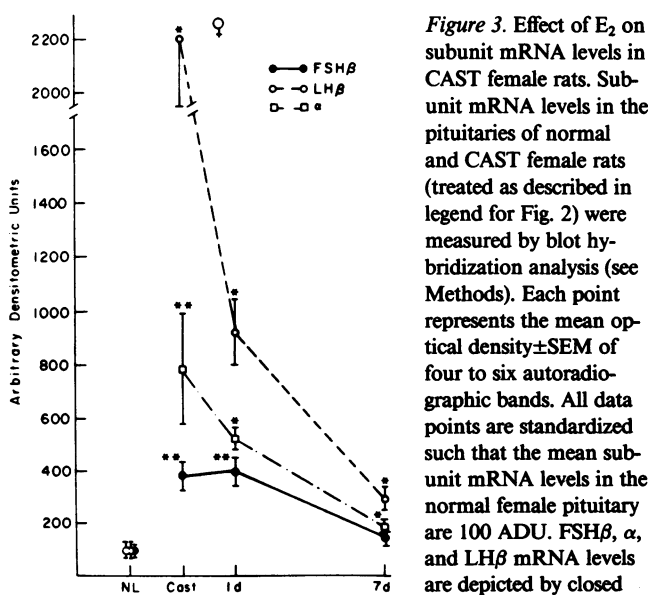


Figure 3. Effect of E₂ on subunit mRNA levels in CAST female rats. Subunit mRNA levels in the pituitaries of normal and CAST female rats (treated as described in legend for Fig. 2) were measured by blot hybridization analysis (see Methods). Each point represents the mean optical density±SEM of four to six autoradiographic bands. All data points are standardized such that the mean subunit mRNA levels in the normal female pituitary are 100 ADU. FSH β , α , and LH β mRNA levels are depicted by closed circles, open squares, and open circles, respectively. Stars represent statistical significance of data points as compared with the values for normal rats: **P* < 0.05 and ***P* < 0.001.

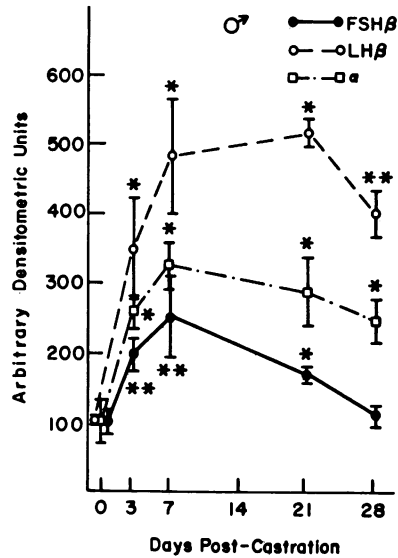


Figure 4. Time course changes in subunit mRNA levels postcastration in male rats. Pituitary subunit mRNAs in CAST male rats were determined by blot hybridization analysis (see Methods) at various time points postcastration. Each point represents the mean density \pm SEM of five to six bands on an autoradiogram (see legend to Fig. 5). All data points are standardized such that the mean subunit mRNA levels in the normal male pituitaries are 100 ADU. FSH β , α , and LH β mRNA levels are depicted by closed circles, open squares, and open circles, respectively. Stars represent statistical significance of data points as compared with the values for normal rats or 0 d postcastration: * $P < 0.05$ and ** $P < 0.001$.

respectively. As seen in the 40-d postcastration males used in the replacement model, there were no statistically significant increases in FSH β mRNA levels at 28 d, although there were minimal, but statistically significant elevations at 3, 7, ($P < 0.001$), and 21 d ($P < 0.05$) postcastration.

Sex steroid replacement. As in the female, both serum FSH and LH increased markedly with orchietomy in male rats. Serum FSH and LH increased three- ($P < 0.001$) and twelvefold ($P < 0.05$), respectively (Table II). With T replacement, serum LH levels declined dramatically so that by 7 d postcastration they were below the levels in intact animals ($P < 0.001$). By comparison, although serum FSH levels declined significantly, the decrements were less striking. By 7 d postcastration, levels were still significantly above normal ($P < 0.001$).

Pituitary LH concentrations also increased with orchietomy such that levels in CAST male rats were threefold ($P < 0.05$) higher than those observed in normals (Table II). With T replacement, pituitary LH levels in CAST males declined markedly but not to the levels seen in intact animals ($P < 0.05$). In contrast, there were no statistically significant elevations in pituitary FSH concentrations with castration. Also, with 7 d of T replacement, pituitary FSH concentrations did not change significantly from normal.

In the male rats, in general, changes in pituitary subunit mRNA levels reflected the changes seen in the serum and pituitary concentrations of FSH and LH. With castration, LH β mRNA levels increased eightfold, whereas α mRNA levels increased fourfold ($P < 0.001$) (Fig. 5). With 1 d of T replacement, LH β mRNA levels increased (1.5-fold), but this rise was not statistically significant. By 7 d of T replacement, LH β mRNA levels had returned to those of normal animals. α mRNA levels also increased after 1 d of T replacement, approximately twofold greater than those of CAST controls ($P < 0.05$), but by 7 d α mRNA levels were not statistically different than those of intact animals. In contrast, there was a minimal rise (1.5-fold) in FSH β mRNA levels with orchietomy, but this rise was not statistically significant. By 1 d of T replacement, FSH β mRNA levels were

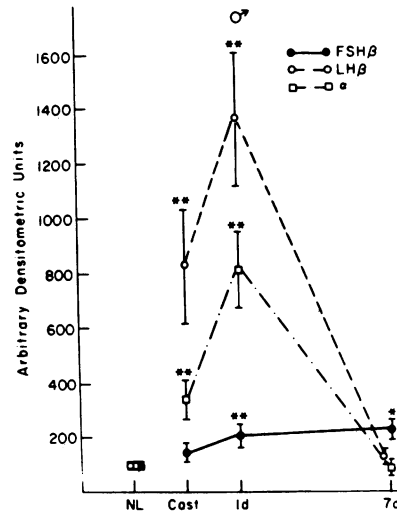


Figure 5. Effect of T on subunit mRNA levels in CAST male rats. Subunit mRNA levels in the pituitaries of CAST male rats treated with T for 0, 1, and 7 d and those of normal males were measured as described in Methods. Each point represents the mean density \pm SEM of six bands on an autoradiogram (see legend for Fig. 3). All data points are standardized such that the mean subunit mRNA levels in the normal male pituitary is 100 ADU. FSH β , α , and LH β subunit mRNA levels are depicted by closed circles, open squares, and open circles, respectively. Stars represent statistical significance of data points as compared with the values for normal rats: * $P < 0.05$ and ** $P < 0.001$.

approximately twofold higher than normals ($P < 0.001$) and remained elevated even after 7 d of T replacement.

Discussion

Discussion

Although much is known about the regulation of FSH at the secretory level, few studies have examined the regulation of the synthesis of FSH β . This study evaluates the differential regulation of FSH and LH by sex steroids at both the synthetic and the secretory levels. We observed a difference in the patterns of changes in FSH β and LH β mRNA levels after castration and subsequent gonadal steroid replacement. In female rats with ovariectomy, LH β and α mRNA levels increased dramatically (twenty- and eightfold, respectively), whereas FSH β mRNA levels rose only modestly (fourfold), although serum levels of both FSH and LH increased markedly. With sex steroid replacement, all three subunits, FSH β , LH β , and α returned toward normal. In male rats, the discrepancy between the pattern of changes in LH β and FSH β mRNAs was more marked. With castration, LH β and α increased eight- and fourfold and returned to normal levels with replacement. However, there were only minimal elevations in FSH β mRNA levels in 40-d CAST male rats despite elevations of serum FSH. Moreover, after 7 d of T replacement FSH β mRNA levels were significantly elevated when compared with normals.

The changes in subunit mRNAs observed in these experiments cannot be explained by changes in cell population alone (19). The percentage of anterior pituitary cells staining for FSH and LH increases from 12 and 9%, respectively, in the intact female rat to 16 and 18%, respectively, 1 mo postovariectomy. In male rats, percentages of FSH- and LH-staining cells increases from 7 and 10%, respectively, to 25% for both 1 mo postorchietomy. Thus, the four- and twentyfold changes in FSH β and LH β mRNA levels observed in the present study in female rats postcastration cannot be solely dependent on modest changes in the gonadotrope population. Similarly, in the male rat, the eightfold rise in LH β mRNA level postorchietomy seen in this

study cannot be explained by the reported 2.5-fold rise in LH-staining cells postcastration. Although FSH-staining cells have been demonstrated to increase up to 3.5-fold 1 mo postcastration, the results of our study show little increase in FSH β mRNA at 40 d postcastration.

It is also important to note that single doses of E₂ and T were administered to female and male rats in this study. These doses were chosen because they are supraphysiologic and had been demonstrated to lower serum FSH and LH levels in previous studies (20–23). Whether other dosage regimens would result in a different pattern of regulation of the gonadotropin subunit mRNAs remains to be established.

There are two remarkable aspects of these findings. First, in both females and males FSH β mRNA levels increased to a much lesser extent by 28 d postcastration than did the LH β mRNA levels. Second, the response of the FSH β mRNA levels to both castration and to sex steroid replacement was markedly different in the males when compared with the females.

Differences in FSH β and LH β steady state mRNA levels could be explained by differential rates of synthesis or degradation or both. LHRH affects the secretion of FSH and LH differentially (24, 25) and has also been shown to increase the synthesis of α and LH β at the pre- (26) and posttranslation (27) levels. Although similar studies have not yet been performed to examine the biosynthesis of FSH β , changes in the pattern of LHRH secretion may be responsible for different rates of synthesis of FSH β and LH β mRNA in the CAST and sex steroid replacement models. The role of other hypothalamic factors, such as the recently described gonadotropin-releasing hormone-associated protein, in the biosynthesis of FSH β and LH β remains to be elucidated (28).

The recently characterized gonadal peptides, the inhibins (6, 7, 29, 30), and FSH-releasing peptides (31, 32) also may contribute to the differential regulation of FSH β and LH β mRNAs in response to castration and sex steroid replacement with potential effects on transcription or RNA stability. The inhibins selectively decrease (6, 7), whereas FSH-releasing peptides selectively stimulate FSH secretion in vitro (32, 33). The interplay between the inhibitory and stimulatory effects of these two classes of gonadal peptides may be important in the synthesis of FSH β mRNA as well as the secretion of FSH and could account for the difference between the changes observed in FSH β and LH β mRNA levels.

The observed sex differences in the patterns of changes in FSH β subunit mRNA levels parallel the changes in the pituitary concentrations of FSH seen in this study. These differences between male and female pituitary FSH content have been described by others (15, 34), but the basis for these differences is unclear. In addition to the increased FSH stores, there also may be increased FSH β mRNA levels in the intact males as compared with those of the female that account for the lack of increases in FSH β mRNA levels in the male with CAST. This hypothesis could not be examined in the present study because the male and female RNA samples were placed on separate blots, and comparisons between absolute amounts of RNA on the two different blots would be invalid. Preliminary data from other studies in our laboratory, however, indicate that indeed the FSH β mRNA levels are higher in intact males as compared with intact females (unpublished data).

The regulation of FSH β mRNA levels by sex steroids in males is complex. Testosterone administration to rats 40 d post-

orchietomy failed to reduce FSH β mRNA levels. Instead, there were slight, but statistically significant increases in FSH β mRNA levels. This was in contrast to the female rats in which the moderately increased castration levels of FSH β mRNA declined with sex steroid replacement. Data from studies of rat anterior pituitary cell cultures indicate that the secretion of FSH is increased (35–37), whereas that of LH is decreased (35) by T in males. In vivo, with T replacement, there may be a balance between changes in the hypothalamic LHRH program resulting in decreased secretion and possibly decreased synthesis of FSH β and direct stimulatory effects of T upon the synthesis of FSH β in the pituitary of male rats. Modest elevations of FSH β mRNA (2–2.5-fold) were observed at early time points postcastration. Sex steroid replacement studies at these earlier time points are needed to further explore the effects of testosterone on FSH β mRNA levels.

In conclusion, in female rats, FSH β mRNA levels are negatively regulated by E₂. The magnitude of these changes is less, however, than that of α or LH β subunit mRNAs. In male rats, the FSH β mRNA levels, like the pituitary content of FSH, are less sensitive to regulation by testosterone. This contrasts strikingly with the testosterone-mediated negative regulation of α and LH β mRNAs. Thus, gonadal steroid hormones affect the synthesis of FSH, at least in part, at the pretranslational level by regulating the steady state levels of subunit mRNAs. Whether this regulation by gonadal steroid hormones occurs directly at the level of the gonadotrope or indirectly via hypothalamic factors, remains to be elucidated. Pituitary cell-culture studies investigating the role of gonadal sex steroids as well as other gonadal and hypothalamic factors on FSH subunit mRNA levels are currently underway in this laboratory.

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