Selective Failure of Androgens to Regulate Follicle Stimulating Hormone β Messenger Ribonucleic Acid Levels in the Male Rat

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FSHβ, as well as LHβ, and α-subunit mRNA levels were examined in the pituitary glands of male rats after sex steroid replacement at various times (7, 28, or 90 days) after orchiectomy. Testosterone propionate, dihydrotestosterone propionate, or 17β-estradiol benzoate (E) were administered daily for 7 days before killing, to assess the role of different gonadal steroids on gonadotropin subunit mRNA levels. Subunit mRNAs were determined by blot hybridization using rat FSHβ genomic DNA, and α and LHβ cDNAs. At all time points, α and LHβ mRNAs increased after gonadectomy and fell toward normal levels with either androgen or estrogen replacement. FSHβ mRNA levels increased variably postcastration: 4-fold at 7 days, 2-fold at 28 days, and 4- to 5-fold at 90 days. Although E replacement uniformly suppressed FSHβ mRNAs, neither testosterone propionate nor dihydrotestosterone propionate administration suppressed FSHβ mRNA levels at any time point after orchiectomy. These data demonstrate that there is a relative lack of negative regulation of FSHβ mRNA levels by androgens in a paradigm in which E administration results in marked negative regulation of FSHβ mRNA levels. Thus, in the male rat, estrogens negatively regulate all three gonadotropin subunit mRNA levels while androgens negative regulate LHβ and α-subunit but fail to suppress FSHβ mRNAs. (Molecular Endocrinology 2: 492–498, 1988)

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INTRODUCTION

We have shown recently that there is a differential regulation of pituitary FSHβ and LHβ mRNA levels by sex steroids in male and female rats (1). In female rats, FSHβ mRNA levels are negatively regulated by estradiol, although the magnitude of the changes is less than those of the LHβ and α-subunits. In male rats FSHβ and mRNA levels, unlike those of α and LHβ, are relatively insensitive to negative regulation by castration and replacement with testosterone. These observations raised two questions. First, is the relative lack of changes in FSHβ mRNA levels in the male time dependent relative to castration (*i.e.* is there a differential sensitivity to androgen feedback at various times after orchiectomy)? Second, what is the underlying etiology for the discordant changes in FSHβ mRNA levels with castration and sex steroid replacement between the male and female rat? Specifically, are there inherent male/female differences in FSHβ gene expression or do estrogens and androgens differentially regulate FSHβ mRNA levels independent of sex?

To address these questions and thus to understand further the role of sex steroids in the regulation of gonadotropin production at a pretranslational level, a castration-replacement model was used in which steroid replacement was examined at various intervals after orchiectomy (7, 28, or 90 days). Testosterone propionate (T), 5α-dihydrotestosterone propionate (DHT), or 17β-estradiol benzoate (E) in supraphysiological doses was administered to male rats to assess

the role of the type of sex steroid on pituitary gonadotropin subunit mRNA levels. Additionally, the relative amounts of mRNA for the α, LHβ, and FSHβ subunits from pools of pituitary glands of intact and gonadectomized male *vs.* female rats were determined.

Our data document that there are modest elevations in FSHβ mRNA levels at 7 and 90 days after castration in the male rat but little elevation at 28 days. Androgen administration at all time points examined consistently failed to regulate FSHβ mRNAs negatively despite suppressing α and LHβ mRNA levels. Estrogen replacement to castrate male rats, however, as was seen previously in the female, resulted in a substantial negative regulation of the FSHβ subunit. These studies demonstrate a marked differential regulation of FSHβ by androgens and estrogen in the male rat.

RESULTS

Seven Days Post Castration

To assess whether one could observe negative regulation of FSHβ mRNA levels in the pituitary gland of the male rat at early times post orchiectomy, the sex steroid replacement paradigm was performed in animals at 7 days after surgical castration. At the secretory level, serum FSH and LH levels increased 4-fold ($P < 0.01$) and 14-fold ($P < 0.01$), respectively (Table 1). With 7 days of T replacement, LH levels declined to below those of intact animals ($P < 0.01$). FSH levels also declined, but less strikingly than LH levels and the decline was not statistically significant.

Table 1. Serum Concentrations of FSH and LH of Intact, Castrate (7, 28, or 90 days), and Castrate Rats Treated with T, DHT, or E for 7 days

	FSH (ng/ml)	LH (ng/ml)
Intact	7.8 ± 0.8	0.6 ± 0.1
Castrate (7 days)	29.0 ± 2.1[a]	9.3 ± 1.2[a]
+T	23.9 ± 2.9[b]	0.3 ± 0.1[b,c]
Intact	8.9 ± 0.4	1.4 ± 0.1
Castrate (28 days)	36.6 ± 2.6[a]	9.2 ± 1.0[a]
+T	21.6 ± 1.3[a,c]	0.6 ± 0.1[a,c]
+DHT	25.8 ± 1.3[a,d]	0.6 ± 0.3[a,c]
+E	17.8 ± 0.6[a,c]	1.8 ± 1.2[b,c]
Intact	9.3 ± 0.6	0.9 ± 0.8
Castrate (90 days)	31.6 ± 3.4[a]	8.4 ± 1.0[a,c]
+T	21.6 ± 2.9[a]	0.5 ± 0.1[a,c]
+DHT	25.4 ± 1.5[b]	0.7 ± 0.1[c]
+E	19.8 ± 1.7[a,d]	2.0 ± 0.1[a,c]

[a] Significance of comparisons to levels in intact animals; $P < 0.01$.

[b] Significance of comparisons to levels in intact animals; $P < 0.05$.

[c] Significance of comparisons to gonadotropin serum levels in castrate animals; $P < 0.01$.

[d] Significance of comparisons to gonadotropin serum levels in castrate animals; $P < 0.05$.

At the level of subunit synthesis, pituitary α and LHβ mRNA levels rose 3.5- ($P < 0.05$) and 10.8-fold ($P < 0.05$), respectively (Fig. 1). At this time point, FSHβ mRNA levels were significantly higher [4.5-fold ($P < 0.05$)] than those in intact animals and animals killed at 28 days but similar to animals killed at 90 days after castration. Although there was striking negative regulation of α and LHβ subunit mRNAs by T ($P < 0.01$), there was no significant decrease in FSHβ levels with androgen administration (P = NS).

Twenty Eight Days Postcastration Experiment

Serum gonadotropin and mRNA levels were measured for intact rats and compared to animals 28 days postcastration that were treated with sesame oil, T, DHT, or E for 7 days before killing. With castration, serum FSH and LH rose 4-fold ($P < 0.01$) and 20-fold ($P < 0.01$), respectively (Table 1). LH levels decreased toward or below intact levels with T ($P < 0.01$), DHT ($P < 0.01$), or E ($P < 0.01$) replacement for 7 days. FSH levels declined less markedly, but to values significantly less than castrate levels for all three steroid treatments [T ($P < 0.01$), DHT ($P < 0.05$), and E ($P < 0.01$)].

At the synthetic level, a dichotomy between the changes observed in FSHβ mRNAs and those of the α and LHβ subunits was demonstrated. Orchiectomy resulted in 5-fold ($P < 0.05$) and 10-fold ($P < 0.05$) increases in α and LHβ mRNA levels, respectively, whereas there was a modest increase [2-fold ($P < 0.05$)] in FSHβ mRNA levels 28 days post castration (Fig. 2A) as previously observed (1). With androgen replacement for 7 days, there were prompt and striking declines in α and LHβ mRNA levels ($P < 0.05$ from Cast) to those seen in intact animals (P = NS from Intact), whereas both T and DHT administration resulted in no significant decline in FSHβ mRNA levels (T, DHT = NS from Cast). Estrogen replacement for 7 days, however, decreased markedly all three subunit mRNAs from those of castrate animals to those observed in intact rats [α ($P < 0.05$); LHβ and FSHβ ($P < 0.01$)].

Three Months Post Castration

By 3 months post orchiectomy, serum FSH and LH levels increased 3.4-fold ($P < 0.01$) and 9.3-fold ($P < 0.01$), respectively, from levels seen in intact animals (Table 1). Hormonal replacement for 1 week with androgens resulted in significant decreases in serum LH levels [(T, $P < 0.01$); (DHT, $P < 0.01$)] but not FSH levels. Estradiol administration, however, produced declines in both gonadotropin values [(FSH, $P < 0.05$) and (LH, $P < 0.01$)]. Again, changes in FSH levels were less marked than those of LH.

At the pretranslational level, as observed at earlier times post castration, the increment in α and LHβ mRNAs were more dramatic, 6-fold ($P < 0.05$) and 9.5-fold ($P < 0.05$), respectively, than the 4.8-fold increment observed in FSHβ ($P < 0.05$) (Fig. 2B). Again, androgen

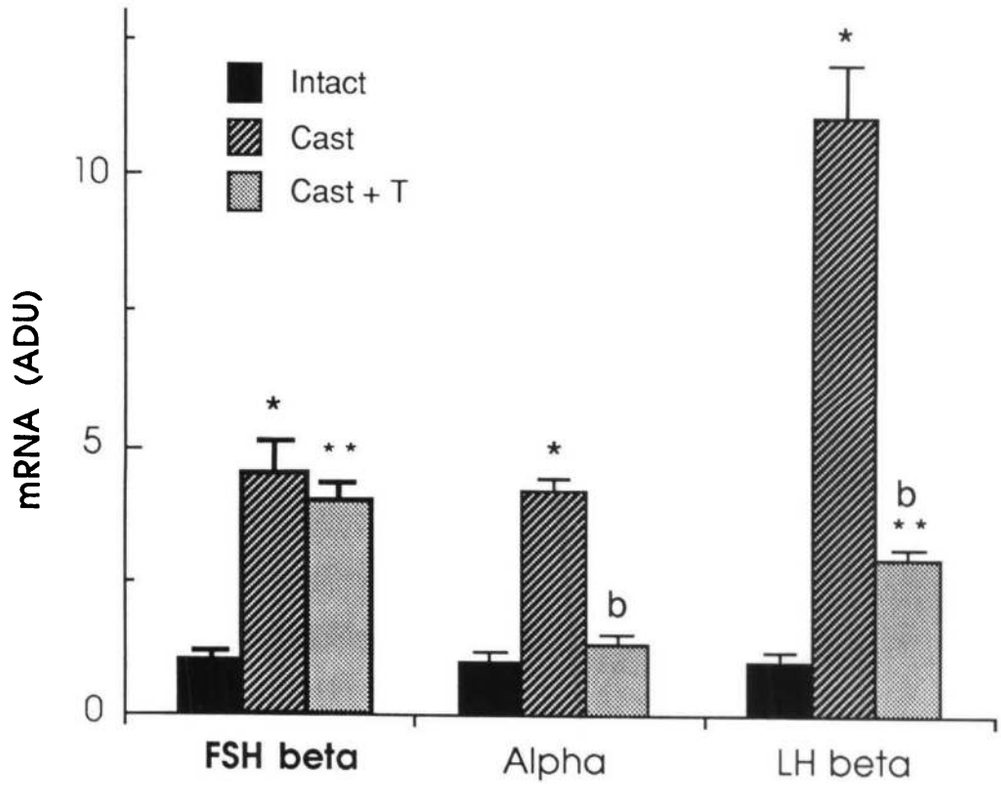

Fig. 1. Effect of T on Subunit mRNA Levels in Male Rats 7 Days Post Castration

Subunit mRNA levels in the individual pituitaries of intact and castrate (Cast) rats were determined by blot hybridization analysis (see *Materials and Methods*). Each column represents the mean optical density ± SEM of three to six autoradiographic bands. All data points are standardized such that the mean subunit mRNA levels in the intact pituitary are 1.0 arbitrary densitometric units (ADU). Symbols represent significance of comparisons of data points with the values for intact animals (*, $P < 0.05$; **, $P < 0.01$) and with those for castrate (Cast) animals (a, $P < 0.05$; b, $P < 0.01$).

replacement resulted in a marked decrement in α [(T, DHT ($P < 0.05$)] and LHβ [(T, DHT ($P < 0.05$)] mRNA levels, whereas there was no change or a slight increase in FSHβ mRNA levels (P = NS from Cast). As observed in the 28-day animals, estrogen administration consistently lowered mRNA levels of all three gonadotropin subunits ($P < 0.05$).

Gonadotropin Subunit mRNA Levels in Male and Female Rats

In an attempt to explore potential etiologies for the apparent lack of negative regulation of FSHβ mRNA levels by androgens in the male rat, we compared pooled samples of total RNA from intact and castrate (1–3 months post Cast) male and female rats. The same blot was probed sequentially with the rat α, LHβ, FSHβ, and mouse β-actin probes. This allowed satisfactory comparison of each subunit mRNA among the different physiological states with normalization. There were similar amounts of steady-state α and LHβ mRNAs in the intact animal which then increased to a variable degree with castration. In the male, both α and LHβ increased 6-fold whereas in the female, α and LHβ increased 8- and 10-fold, respectively (data not shown). There was a striking discordance, however, in the basal mRNA levels of FSHβ between intact male and female rats. Female rats were observed to have only approximately 30% of the FSHβ mRNA levels seen in intact male rats (Fig. 3). With castration, FSHβ mRNA levels increased 6- to 8-fold in the female and up to 2- to 3-fold in the male, such that the castrate levels were comparable between the two sexes.

DISCUSSION

In this report, we examined the dissociation between the regulation of FSHβ and LHβ subunits by sex steroids at the pretranslational level in the male rat. The *in vivo* sex steroid replacement model was chosen because it has been well characterized by us (1–3) and others (4–9) to examine the regulation of gonadotropin subunit mRNA levels. Various sex steroids were employed to confirm our previous observations of the effects of T replacement, as well as to examine the effects of an E replacement regimen in the castrate male, previously shown to result in striking negative feedback of FSHβ mRNA levels in the female (1). A subset of animals treated with DHT was included to dissect the role of aromatization in sex steroid feedback.

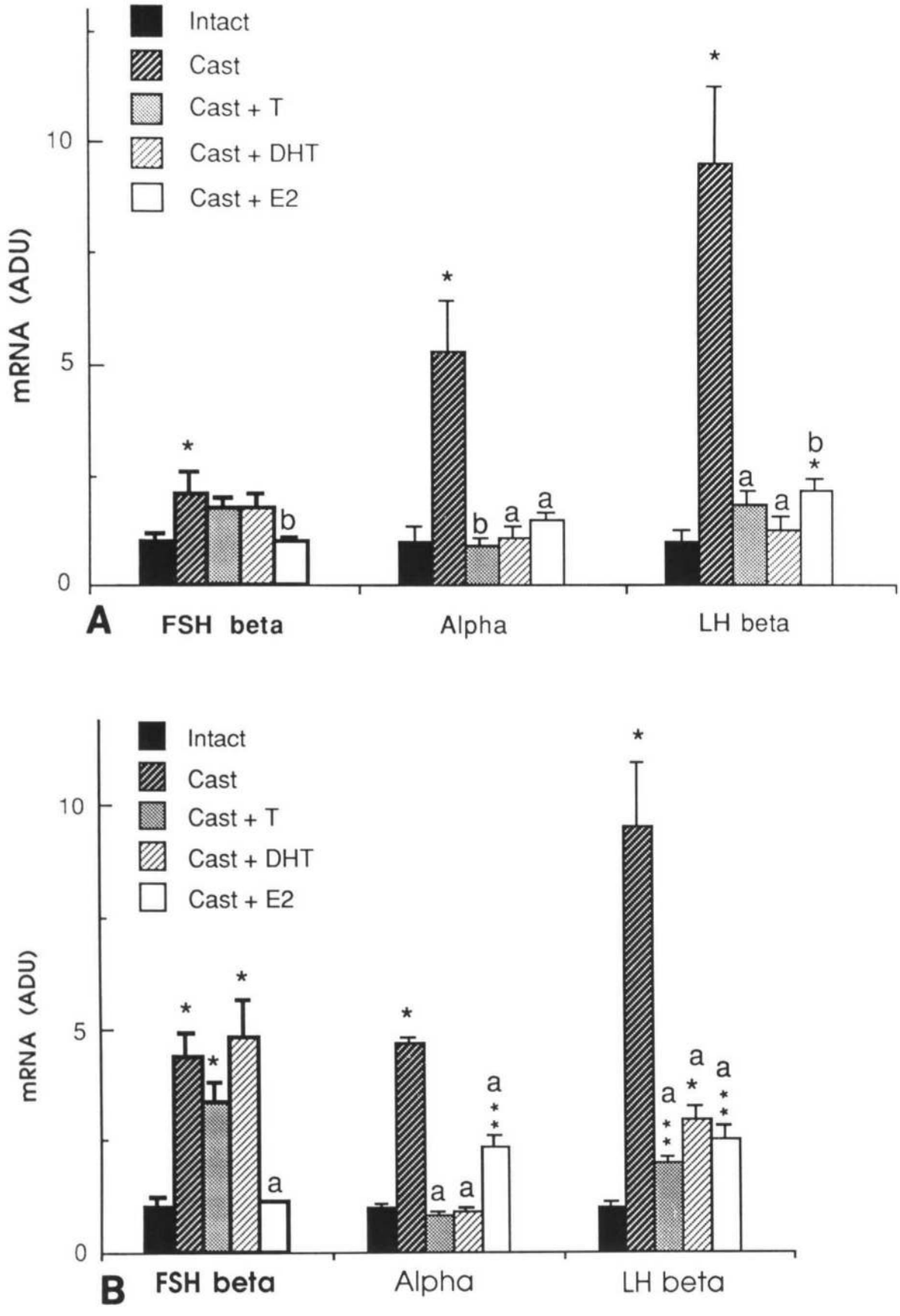

Fig. 2. Effect of Gonadal Steroid Replacement on Subunit mRNA Levels in Male Rats 28 and 90 Days Post Castration

Male rats, 1 month (A) or 3 months (B) post orchiectomy were replaced with 0 or 7 days of T, DHT, or E. Subunit mRNA levels were determined (see *Materials and Methods*). Each column represents the mean optical density ± SEM of three to six autoradiographic bands. All data points are standardized such that the mean subunit mRNA levels in the intact pituitary are 1.0 ADU. Symbols represent significance of comparisons of data points with the values for intact animals (*, $P < 0.05$; **, $P < 0.01$) and with those for castrate (Cast) animals (a, $P < 0.05$; b, $P < 0.01$).

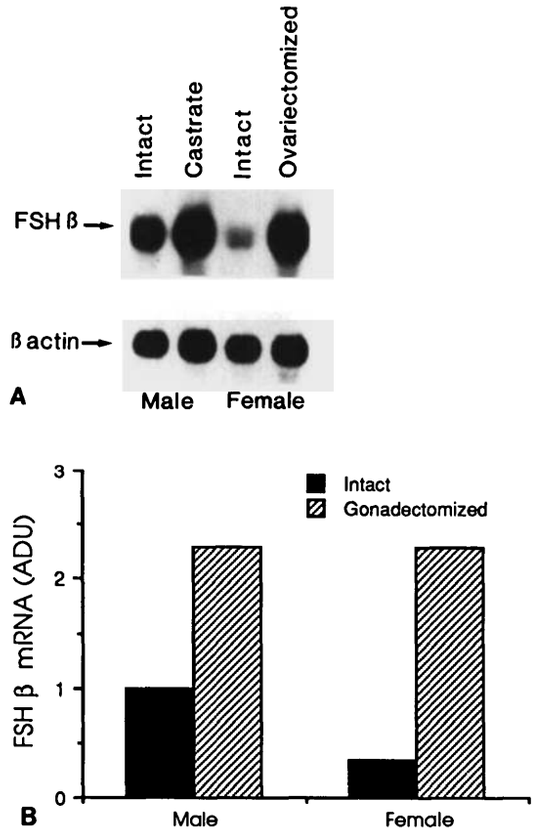

Fig. 3. Intact and Castrate FSHβ and mRNA Levels

A, Northern blot hybridization analysis. A radiolabeled genomic fragment encoding the rat FSHβ subunit was hybridized to total cellular RNA from pools of pituitary glands of intact and Cast (since ♀ + ♂) (1–3 months) rats as described in *Materials and Methods*. Each lane contains 10 μg RNA. The same blot was also hybridized with β-actin (*lower bands*) to correct for total RNA loaded. Film exposure time was 24 h using Kodak-XAR film and an intensifying screen at −70 C. B, A comparison of pituitary FSHβ mRNA levels in male and female rats. Subunit mRNA levels in pools of pituitaries of intact and Cast rats (1–3 months after castration) were determined by blot hybridization analysis (see *Materials and Methods*). All data points are standardized such that the FSHβ mRNA levels in the intact pituitary are 1.0 ADU.

Sex steroid dosages employed were supraphysiological but are those routinely used to suppress reliably serum gonadotropins to those approximating the intact animal in acute or chronically castrate animals (10–15). In our experiments, 7 days of androgen replacement variably suppressed serum FSH levels in the different groups of castrate animals, while LH levels were uniformly suppressed by each of the gonadal steroids. Both at the secretory as well as the pretranslational level, androgens were less effective than estrogen in effecting negative feedback of FSH in the male rat.

Hormone replacement experiments of castrate animals at various intervals after orchiectomy were performed to examine potential changes in the sensitivity of FSHβ mRNA levels to sex steroid feedback in the male at varying times post castration. FSHβ mRNA levels were modestly elevated at 7 days (4.5-fold) and 90 days (4.8-fold) post castration when compared to intact levels. This suggests that some component of the gonad (either androgen, estrogen, or gonadal peptide) that confers negative feedback has been removed. Yet at all times examined after orchiectomy, androgen replacement failed to result in a decline in FSHβ subunit mRNA levels.

Our data demonstrate that, in the male rat, androgen replacement fails to negatively regulate FSHβ mRNA levels in a paradigm in which α and LHβ mRNAs are markedly suppressed. Estrogen administration in the male, however, does result in a significant fall in FSHβ mRNA levels. This suggests that the discrepancy previously documented in FSHβ mRNA regulation (1) between males and females may be explained by the differences in endogenous hormonal milieu. The lack of differences between the mRNA levels of T and DHT replaced groups suggests that the conversion of testosterone to estradiol in the T-treated animals must have been inadequate to effect a decrement in FSHβ mRNA levels. Whether similar results would be obtained with physiological sex steroid replacement, or for longer or shorter intervals of steroid administration remains to be examined.

To examine further the male-female differences in the regulation of FSHβ, we directly compared FSHβ mRNA levels in intact male and female rats and observed a considerable difference in the basal FSHβ mRNA levels between the sexes. These data are consistent with differences previously observed in the pituitary content of FSH between the sexes (1, 2, 16–18). Higher basal FSHβ mRNA levels would be expected in the intact male compared to female rat if androgens are less effective than estrogens in negatively regulating FSHβ mRNA levels. In addition, the higher basal FSHβ mRNA values in intact males might partially explain the less striking increments measured in our assay post castration. However, the fact that estrogen administration clearly suppresses mRNA levels in the male as well as the female confirms that the assay used is sensitive enough to reliably detect changes of FSHβ mRNA levels in the male.

Various hypotheses may explain the lack of androgen regulation of FSHβ in the male rat. Perhaps, in the male, the gonadal inhibins (19–25) play a greater role in the negative regulation of FSHβ mRNA levels, whereas, in the female, sex steroids may play a more dominant role. The fact that FSHβ mRNA levels rise modestly post castration yet cannot be suppressed by androgen replacement alone is consistent with this hypothesis. Administration of the purified or genetically engineered gonadal peptides would address this question.

It is possible, however, that androgens do not regulate FSHβ mRNA levels in either sex despite marked regulation of α and LHβ subunit mRNAs. Studies in which various androgens are administered to ovariectomized rats are in progress to address whether the lack of negative regulation of FSHβ mRNAs is sex specific or characteristic of the class of gonadal steroids. Perhaps androgens have no direct effects upon FSHβ mRNA levels but act only via conversion to

estradiol. In our study, there were no differences seen between the T and DHT-replaced animals; however, the steroid dosage or length of administration may have been suboptimal to demonstrate a differential effect due to aromatization.

Finally, androgens may have conflicting feedback effects upon the hypothalamus and pituitary. Reports of pituitary cell culture experiments suggest a positive feedback effect of testosterone on FSH secretion (26–28). No data are available concerning FSH synthesis. Investigators also have demonstrated negative feedback effects of androgens at the level of the hypothalamus on gonadotropin secretion. In these studies, androgens have been shown to decrease GnRH-induced LH pulse frequency (29, 30). These opposing effects at different loci within the hypothalamic-pituitary-gonadal axis may contribute to an apparent lack of suppression of FSHβ mRNA levels by androgens in the male. *In vitro* cell culture experiments are in progress in this laboratory to examine direct steroid feedback effects on gonadotropin subunit expression.

In conclusion, in both male and female rats, FSHβ mRNA levels are negatively regulated by E along with the α and LHβ subunit mRNAs. In males, there appears to be some loss of negative feedback at the pretranslational level with removal of the testes; however, androgen administration for 7 days is unable to lower FSHβ mRNA levels towards those in intact animals. Further studies in the female and use of *in vitro* cell culture techniques are needed to elucidate the underlying etiologies for the differential regulation of FSHβ by gonadal steroids in the rat.

MATERIALS AND METHODS

Experimental Design

Adult male Sprague-Dawley rats (CD strain, 200–225 g; Charles River Breeding Laboratories, Inc.; Wilmington, MA) were used in all experiments. Hormone replacement experiments were performed at various time intervals after surgical castration. Six animals were used for each experimental group. There were three to five experimental groups for each time point: intact animals (I), and castrated animals which received seven daily injections of sesame oil (Cast), T (500 μg/100 g BW), DHT (500 μg/100 g BW), or E (10 μg/100 g BW). Groups of castrate animals underwent sex steroid replacement at 28 or 90 days post orchiectomy. An additional group of animals (7 days post castration) received only oil or T replacement. All animals were then killed by decapitation and trunk blood was collected for serum gonadotropin determination by RIA to compare changes at the secretory level with those at the pretranslational level. Pituitaries were recovered quickly and stored in liquid nitrogen. Also, intact and castrate male and female rats (1–3 months post castration) were killed and total RNA isolated from pooled pituitary samples.

RIA of Gonadotropins

Serum levels of FSH and LH were determined by RIA on trunk blood from individual animals using reagents from the NIDDK as described (1, 16).

DNA Probes

The rat α synthetic oligonucleotide probe, the rat LHβ, and mouse β-actin cDNA, and the rat FSHβ genomic DNA probes have been described previously (1, 2). The synthetic α primer was labeled using a 5′-end labeling technique (31) to a specific activity of 0.5–1.0 × 10^8 cpm/μg DNA, and the cDNA and genomic fragments were labeled using random primer translation (32) to achieve a specific activity of 0.5–1.0 × 10^9 cpm/μg/DNA.

Subunit mRNA Determinations

Six individual pituitaries were processed for each of the five experimental subgroups. Total RNA was extracted from individual pituitaries as previously reported (1, 2). Four Northern blots were prepared. Ten micrograms (OD_{260}) of total RNA from each sample were subjected to electrophoresis and diffusion blotted onto nitrocellulose (33). Individual blots were prepared for each of the replacement time points post castration (7, 28, 90 days) and a fourth blot included total RNA from pooled samples of intact and castrate male and female rats in order to make a direct comparison of gonadotropin subunit mRNA levels between the sexes. Each blot was sequentially hybridized with the α, LHβ, FSHβ, and finally, the β-actin probes using conditions outlined previously (1, 2). Blots were washed (1), subjected to autoradiography, and the band densities were determined by scanning densitometric analysis.

Standardization of Data

The amount of RNA in each lane of each blot (10 μg by OD_{260}) was internally standardized within a blot by assessing the amount of β-actin mRNA per lane and correcting the α, LHβ, and FSHβ mRNA levels accordingly, as described previously (1, 2).

Statistical Analysis

The Wilcoxon rank sum test (Mann-Whitney variation) was employed for analysis of mRNA data. A *t* test for independent samples was used to assess the statistical significance of changes in the serum levels of gonadotropins.

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