The regulation of FSHβ transcription by gonadal steroids: testosterone and estradiol modulation of the activin intracellular signaling pathway

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Burger LL, Haisenleder DJ, Wotton GM, Aylor KW, Dalkin AC, Marshall JC. The regulation of FSHβ transcription by gonadal steroids: testosterone and estradiol modulation of the activin intracellular signaling pathway. Am J Physiol Endocrinol Metab 293: E277–E285, 2007. First published April 3, 2007; doi:10.1152/ajpendo.00447.2006.—Recent reports suggest that androgens increase FSHβ transcription directly via the androgen receptor and by modulating activin signaling. Estrogens may also regulate FSHβ transcription in part through the activin system. Activin signaling can be regulated extracellularly via activin, inhibin, or follistatin (FS) or intracellularly via the Smad proteins. We determined the effects of androgen and estrogen on FSHβ primary transcript (PT) concentrations in male and female rats, and we correlated those changes with pituitary: activin βB mRNA, FS mRNA, the mRNAs for Smads2, -3, -4, and -7, and the phosphorylation (p) status of Smad2 and -3 proteins. In males, testosterone (T) increased FSHβ PT two- to threefold between 3 and 24 h and was correlated with reduced FS mRNA, transient increases in Smad2, -4, and -7 mRNAs, and a six- to 10-fold increase in pSmad2, and activin βB mRNA was unchanged. In females, T also increased FSHβ PT twofold and pSmad2 threefold but had no effect on activin βB, FS, or the Smad mRNAs. Androgen also increased Smad2 phosphorylation in gonadotrope-derived αT3 cells. In contrast, estradiol had no effect on FSHβ PT but transiently increased activin βB mRNA and suppressed FS mRNA before increasing FS mRNA at 24 h and increased Smad2, -3, -7 mRNAs and pSmad2 threefold. In conclusion, T acts on the pituitary to increase FSHβ PT in both sexes and modulates FS mRNA, Smad mRNAs, and/or Smad2 phosphorylation. These findings suggest that T regulates FSHβ transcription, in part, through modulation of various components of the activin-signaling system. Estradiol more weakly increases activin βB mRNA and suppressed FSHβ mRNA at 24 h and increased Smad2, -3, -7 mRNAs and pSmad2 threefold. In conclusion, T acts on the pituitary to increase FSHβ PT in both sexes and modulates FS mRNA, Smad mRNAs, and/or Smad2 phosphorylation. These findings suggest that T regulates FSHβ transcription, in part, through modulation of various components of the activin-signaling system.

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regulate gene activity (for review, see Ref. 18). Additionally, activin intracellular signaling can be regulated by "inhibitory" Smad7, which binds to the type I receptor and prevents Smad2 or -3 phosphorylation or hastens their degradation via recruitment of phosphatases.

The objective of this study was to examine androgen and estrogen regulation of FSHβ gene transcription and whether it involves the availability of bioactive activin or the Smad intracellular signaling pathway. To this end, we characterized changes in activin βB mRNA, FS mRNA, Smad2, -3, -4, and -7 mRNA expression, and Smad2/3 phosphorylation in pituitaries from gonadectomized GnRH-deficient male and female rats treated with physiological levels of T and E2.

MATERIALS AND METHODS

Animal models. Adult (225–250 g) male and female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used for all experiments. Rats were housed in a light- (lights on 0500–1700) and temperature-controlled (25°C) room and allowed access to food and water ad libitum. All surgeries were performed under isoflurane (2.5% isoflurane, balance O2; ISO-THESIA, Vetus Animal Health, Burns Veterinary Supply, Westbury, NY) anesthesia. At the completion of experiments, rats were euthanized by decapitation under isoflurane anesthesia. Trunk blood was collected for serum hormone measurements. Pituitaries were collected and bisected, and then one-half was processed immediately for protein and the other half snap-frozen in liquid nitrogen and stored at −70°C until RNA was extracted. The University of Virginia Animal Care and Use Committee approved the animal experimentation described within this report.

To isolate the effects of steroids on the pituitary, we employed a gonadectomized (GDX) GnRH antagonist-treated animal model as previously reported (9, 10, 39). Groups of male and female rats were GDX (n = 5–7/group) and given the water-soluble GnRH antagonist LRF-147 (100 µg/0.5 ml, 0.1% BSA-0.9% NaCl, sc) every 12 h. Four days after gonadectomy, male rats received two T implants each containing a 20-mm column of crystalline T that results in male T levels (~6 ng/ml after 24 h) (10). Female rats received one T implant containing a 5-mm column of crystalline T that results in female T levels (~300 pg/ml after 24 h) (51). Groups of rats were then killed 0, 3, 8, and 24 h after the onset of T treatment. To determine whether the effects of T are androgen specific, one group was also treated for 24 h with dihydrotestosterone (DHT), as previously described (25). Pituitary FSHβ PT and activin βB, FS, Smad2, -3, -4, and -7 mRNAs, Smad phosphorylation, serum gonadotropins, and T were measured.

To determine whether T alters Smad phosphorylation in gonadotropes, we utilized the gonadotrope derived αT3 cells. αT3 cells were plated onto 32-mm culture dishes and grown until confluent in DMEM [containing phenol red, l-glutamine, 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml)]. Twenty-four hours before androgen treatment, the medium was replaced with phenol red-free DMEM with charcoal-stripped FBS (5% FBS). The next day, the medium was replaced with serum-free, phenol red-free DMEM for 4 h, and then cells were treated for 8 h with 100 ng/ml T or vehicle (0.2% ethanol; n = 6 wells/treatment). As a positive control for Smad phosphorylation, one to two separate wells were also treated with 30 ng/ml recombinant human activin A (R&D Systems, Minneapolis, MN) for 1 h. Upon completion, cells were washed with phosphate-buffered saline, lysed, and protein collected to determine Smad2/3 phosphorylation. Experiments were repeated at least two times to confirm results.

Because estrogen is the dominant gonadal hormone in females, we also investigated the effects of physiological levels of E2 on the regulation of FSHβ in female rats. Groups of female rats (n = 5–6/group) were ovarietomized (O VX) and then treated for 4 days with GnRH antagonist (100 µg LRF-147, 12 h). Rats then received one silicone implant containing 25-mm column of 1 mg/ml E2 in sesame oil (Sigma); silastic tubing 1.6 mm id, 3.2 mm od ( Dow Corning, Midland, MI) that resulted in proestrus E2 levels (~50 pg/ml). Rats were then killed 0, 3, 8, and 24 h after the onset of E2 treatment. Pituitary FSHβ PT and activin βB, FS, Smad2, -3, -4, and -7 mRNAs, Smad phosphorylation, serum gonadotropins, and E2 were measured.

Measurement of serum hormones, RNA preparation, the mRNAs for activin βB, FS, and Smad mRNAs, and FSHβ PT. Serum LH and FSH were measured by RIA using the standards NIDDK RP-3 for LH and NIDDK RP-2 for FSH (National Hormone and Pituitary Program). The sensitivities for the LH and FSH assays are 0.09 and 0.8 ng/ml, respectively. The coefficients of variation are 4.7 and 13.5% (intra- and interassay) for LH and 4.6 and 14.4% for the FSH assay. T and E2 were measured by RIA using kits provided by Diagnostic Products (Los Angeles, CA) and Diagnostic Systems Laboratories (Webster, TX), respectively. The sensitivities and coefficients of variation for the assays are 0.1 ng/ml, 5.0%, and 8.2% (intra- and interassay) for T and 10 pg/ml, 5.2%, and 12.1% for E2.

Total pituitary RNA was extracted using the tri-reagent guanidium method (13). Residual genomic DNA was removed by treatment with 1 U RNase Free DNase I (µg RNA (Roche Molecular Biochemicals, Indianapolis, IN) at 37°C for 1 h. RNA preparations were confirmed to be DNA free by PCR in the absence of a preceding RT reaction. FSHβ PT and FS mRNA were measured by quantitative RT-PCR assays, as previously described (14, 28). The mRNA for activin βB and the Smads were determined by real time RT-PCR using an iCycler IQ (Bio-Rad, Hercules, CA) and the QuantO-Tech SYBR Green RT-PCR Kit (Qiagen, Valencia, CA). The real-time assay for activin βB mRNA is based on the amplicon from our previously described quantitative RT-PCR assay (8). The areas amplified for Smad2, -3, -4, and 7 mRNAs are based on previous real-time PCR assay reported by Drummond et al. (19). Assay conditions were optimized to generate a single PCR product as determined by melt curves and agarose gel electrophoresis. The primers used were activin βB forward (FWD) 3′-GCCAGCGGATCATGTTTTAT-5′, reverse (REV) 3′-ACTC-TACCTCTTGGGTAATTGAG-5′; Smad2 FWD 5′-TATCATC-CCAGAAACACCCA-3′, REV 5′-CAGGCGACTCCCTTCTC-3′; Smad3 FWD 5′-GGGCGTACAGACCTTGTGGA-3′, REV 5′-TG-TAGTCTACAGGAGGGGGGA-3′; Smad4 FWD 5′-GCAAGAT-GTCCTACAGGCCCCTCA-3′, REV 5′-CGATCTCCTTCCAGAAGG-ATCCA-3′; and Smad7 FWD 5′-GACCCCCCATACCTTATGT- CGA-3′, REV 5′-CTTTGCCTCCTACTCTGTACCA-3. PCR product identity was confirmed by DNA sequencing. Unknown samples were measured using 10–100 ng RNA against an external standard curve. All assays, including standards, were measured in triplicate. All samples from a study were measured in the same assay. Mean intra-assay coefficients of variation are 12.1, 14.8, 12.8, 12.9, and 13.8% for activin βB and Smad2, -3, -4, and -7, respectively.

Pituitary protein preparation Western immunoblot assays. For protein isolation, hemi pituitaries were homogenized in tissue lysis buffer (50 mM HEPES, 100 mM NaCl, 2 mM EDTA, 1% NP-40, and protease and phosphatase inhibitor cocktails (P8340 and P5726, respectively, with stocks considered ×100; Sigma, St Louis, MO)). Pituitary protein lysates (50 µg/rat sample, 30 µg/αT3 cell lysate) were resolved by electrophoresis (12.5% SDS PAGE) and then transferred to nitrocellulose filters. Receptor-mediated Smad2 and -3 phosphorylation and total protein were measured in Western immunoblot assay. For phosphorylated Smad2 and -3, primary antibodies (rabbit) were obtained from Cell Signaling Technology (Beverly, MA); each antibody recognizes a protein of 58 kDa apparent molecular weight. The secondary antibody was horseradish peroxidase-conjugated goat anti-rabbit (Upstate Biotechnology, Lake Placid, NY). As a positive control for Smad2 and -3 phosphorylation, each sample from a study were measured in the same assay. Mean intra-assay coefficients of variation are 12.1, 14.8, 12.8, 12.9, and 13.8% for activin βB and Smad2, -3, -4, and -7, respectively.
The effects of T in male and female rats on pituitary FSHβ PT, activin βB mRNA, and FS mRNA are shown in Fig. 1. T administration induced a rapid (<3 h) and sustained (through 24 h) increase in FSHβ PT in both male and female rats, with the greater magnitude of increase (3-fold) in males vs. females (2-fold). Activin βB mRNA levels tended to decline after T in both sexes but did not reach significance. As previously reported (10), the increase in FSHβ PT in male rats was accompanied by a 50–60% reduction in FS mRNA. However, FS mRNA did not change after T in females.

The effects of T on Smad mRNAs are shown in Fig. 2. T increased the mRNAs for Smad2, -4, and -7 at 8 h (vs. 0-h controls) in male rats but had no effect on Smad mRNA expression in female rats. The effects of T on FSHβ PT in males have previously been shown to be androgen specific (10), and the effects of the nonaromatizable androgen DHT on female rats are shown in Table 1. Twenty-four hours of DHT increased serum FSH, FSHβ PT, and the mRNAs for Smad3, -4, and -7 and suppressed FS mRNA at 24 h. The differences in results between T and DHT in females are likely due to androgen dosage.

To determine whether T modulates Smad activity, we used Western immunoblot assays to measure pSmad2 and -3 as well as tSmad2/3, and results are shown in Fig. 3. In light of the rapid induction of FSHβ PT by T (2- to 3-fold increase <3h), we measured Smad phosphorylation between 0 and 8 h. At baseline, pSmad3 was barely detectable in male or female pituitary protein samples, and there did not appear to be any change with T treatment (Fig. 3A). In contrast, pSmad2 signal was easily detected and showed a significant induction by T. Between 3 and 8 h, pSmad2 increased 10- and sixfold, respectively, in male pituitaries, whereas in females the increase was not as large (1.5- to 2.5-fold) and peaked later at 8 h. T induced a small and transient increase in tSmad2/3 in female rat.
pituitaries at 3 h and had no effect on males. To control for protein loading and transfer, GAPDH levels were also measured for all blots, and no differences were observed between samples.

To determine whether androgen-induced increases in Smad phosphorylation occur in gonadotropes, we treated /H9251 T3 cells with 100 ng/ml T for 8 h. Similarly to results in rats, T increased Smad2 phosphorylation 3.9-fold vs. vehicle-treated controls after 8 h (P < 0.05 vs. controls; Fig. 4). As in whole pituitary lysates, pSmad3 protein was very low basally and did not change with androgen treatment (results not shown). tSmad2/3 levels were also constant after T treatment.

**DISCUSSION**

The present results extend previous data and show that physiological T concentrations act directly on the pituitary to increase FSH PT transcription in both male and female rats. Furthermore, the effects of T are androgen specific, as nonaromatizable DHT increased FSH PT and E2 did not. Potentially, androgens could increase FSH PT transcription directly via the classical ligand-bound steroid receptor-signaling pathway, by enhancing the activity of other signaling systems (e.g., activin/Smad), or by a combination thereof. Spady et al. (41) and Thackery et al. (45) have reported that androgen increased the

**Table 1.** Mean levels of serum FSH, FSH PT, and the mRNAs for FS, Smad2, Smad3, Smad4, and Smad7 in OVX female rats treated with GnRH antagonist only (0–h controls), T, or DHT for 24 h

<table>
<thead>
<tr>
<th></th>
<th>FSH</th>
<th>FSH PT</th>
<th>FS mRNA</th>
<th>Smad2 mRNA</th>
<th>Smad3 mRNA</th>
<th>Smad4 mRNA</th>
<th>Smad7 mRNA</th>
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<tr>
<td>0 h</td>
<td>4.9±0.5a</td>
<td>100.0±8.4a</td>
<td>100.0±8.6a</td>
<td>100.0±9.4a</td>
<td>100.0±2.1a</td>
<td>100.0±6.3a</td>
<td>100.0±10.3a</td>
</tr>
<tr>
<td>24-h T</td>
<td>5.7±0.4a</td>
<td>176.0±14.8b</td>
<td>104.7±16.2a</td>
<td>102.8±11.6</td>
<td>112.7±13.5a</td>
<td>107.5±6.3b</td>
<td>110.0±9.9</td>
</tr>
<tr>
<td>24-h DHT</td>
<td>7.4±0.1b</td>
<td>249.1±21.2b</td>
<td>58.7±6.4b</td>
<td>116.3±17.4</td>
<td>157.8±15.6a</td>
<td>164.6±27.6b</td>
<td>132.3±12.0</td>
</tr>
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Data are presented as means ± SE. PT, primary transcript; FS, follistatin; OVX, ovariectomized; GnRH, gonadotropin-releasing hormone; T, testosterone; DHT, dihydrotestosterone. Serum FSH is presented in ng/ml and all others as %untreated (0–h) controls (n = 5–6/group). Means with different letters are significantly different P < 0.05.

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activity of ovine and mouse FSHβ promoter-reporter constructs transfected into pituitary-derived LβT2 cells. Additionally, they identified three and six AREs in the ovine and murine FSHβ promoters, respectively, and mutations in the most distal ARE in both, as well as most proximal ARE in the sheep, rendered the promoter constructs androgen insensitive. These data indicate that androgen acts, at least in part, through binding to its own receptor and interacting with androgen-responsive gene sequences to stimulate FSHβ transcription.

However, data reported here as well as in a number of earlier studies (7, 10, 30, 41) indicate that androgen may also regulate FSH indirectly via modulating the activity of activin. In male rats, the T-induced increase in FSHβ PT is temporally correlated with a suppression of FS mRNA, suggesting that T may also act indirectly by increasing the bioavailability of pituitary activin. Similarly, the administration of exogenous FS to pituitary cell cultures has been shown to block androgen-induced FSH secretion (7, 30). Also, Spady et al. (41) found that FS treatment or mutation of an activin-responsive Smad binding element (SBE) in the ovine FSHβ promoter abrogated the induction of the promoter by androgen. In contrast, our earlier studies in primary rat pituitary cells (10) revealed that, despite addition of exogenous FS, T treatment still increased FSHβ transcription. The differences between these two studies are likely due to experimental methodologies. Specifically, we cotreated rat pituitary cells with 30 ng/ml recombinant humanFS and androgen, whereas Spady et al. pretreated LβT2 cells for 20 h with 250 ng/ml rhFS before treatment with androgen ±FS. Therefore, in our paradigm the activin intracellular signaling system was likely undisturbed when cells were treated with androgen, which may account for why we still observed an increase in FSHβ PT, whereas they found that androgen-

Fig. 3. The effects of T on phosphorylated Smad 2, phosphorylated Smad 3, and total Smad 2 and -3 (pSmad 2, pSmad 3, and tSmad 2/3, respectively) in pituitary protein collected from GnRH-deficient gonadectomized male and female rats (details in Fig. 1). A: representative Western blots of pituitary protein in male rats treated for 0, 3, or 8 h with T and immunostained for pSmad 2, pSmad 3, tSmad 2/3, and GAPDH. Protein amounts are 50 μg/lane for pituitary lysates and 10 μg/lane for αT3 control lysates. B: changes in Smad 2 phosphorylation and tSmad 2/3 in both male and female rats were quantified by densitometry and expressed as %0–h (±SE) controls. Points with different letters are significantly different (P < 0.05).
induced FSHβ promoter activity was highly dependent on activin and presumably its intracellular signaling system. Accordingly, we aimed to determine whether androgen affected components of the activin-signaling system and whether those changes were correlated with FSHβ transcription.

We found that T rapidly increased FSHβ PT in both male and female rats but had sex-specific effects on FS and Smad mRNAs. In male rats, T suppressed FS mRNA and transiently increased the mRNAs for Smad2, -4, and -7. In contrast, in female rats a lower T dose increased FSHβ PT but had no effect on FS or Smad mRNA expression. The differences in FS and Smad mRNA expression between the sexes may reflect androgen dosage, as female rats treated with DHT responded in a fashion similar to males, with a larger increase in serum FSH and FSHβ PT, suppression of FS mRNA, and increased Smad3 and -4 mRNAs. However, it is important to note that in female rats T stimulation of FSHβ transcription occurs in the absence of androgen-specific changes in the activin-signaling pathway.

Because the changes in FS and Smad mRNAs are not required for androgen induction of FSHβ transcription, it is possible that androgens could modulate inhibitor Smad7 gene expression, which is known to be involved in negative feedback of activin intracellular signaling (18). Smad7 mRNA is present in both normal pituitary cells and gonadotrope cell lines and is stimulated by activin, a response that can be blocked by FS (5). Overexpression of Smad7 protein in LβT2 cells disrupts activin-induced increases in FSHβ gene expression (4, 20). However, we observed only modest changes in Smad7 mRNA concentration in male rats, and no differences were seen in females. Thus, alterations in the endogenous inhibitor of activin signaling, Smad 7, likely do not serve to modulate androgen action. There are no reports of hormone-modulated changes in Smad2, -3, or -4 mRNA in gonadotropes, but in other cell types TGFβ family members have been reported to increase these RNAs in a feed-forward mechanism to regulate the sensitivity of their signaling pathways (1). Overexpression of Smad3 alone or in combination with Smad4 increases FSHβ promoter activity in LβT2 cells (4, 20, 22, 29, 43, 44). Smad2 overexpression also increases FSHβ promoter activity, but only in combination with Smad4 (4, 20, 22, 29, 43). Additionally, FSHβ promoter activity is attenuated when Smad2 or -3 protein is suppressed (4, 20, 29).

Alternatively to changes in Smad gene expression, androgens could regulate the FSHβ gene via modulation of Smad (-2
and/or -3) phosphorylation. In pituitary cells, activin-induced increases in FSHβ transcription have been correlated to increased phosphorylation of Smad2 and -3 (4, 20). We investigated the effects of T on phosphorylation of the COOH-terminal SSXS motif of Smad2 and -3, which is regulated by the type I receptor. Despite data suggesting that Smad3, in combination with Smad4, plays the major role in transmitting the activin signal for the FSHβ gene (22, 43, 44), we were unable to detect pSmad3 signal either basally or after androgen exposure in pituitary lysates or αT3 cells. This did not appear to reflect limitations in our Western blot assay, as we had no trouble observing activin-induced Smad3 phosphorylation in αT3 cells. Therefore, we conclude that basal pSmad3 levels in the rat pituitary are low and unaffected by T. In contrast, pSmad2 levels were easily detected and increased after T administration; Smad2 phosphorylation increased more rapidly and to a greater degree in males vs. females, which again may reflect androgen dosage. One limitation of in vivo studies is that the pituitary is composed of multiple cell types, and it cannot be determined by Western blotting whether androgen induction of Smad2 phosphorylation is occurring in gonadotrope cells. To address this issue, we treated gonadotrope-derived αT3 cells with T and found that androgen also increases Smad2 phosphorylation in these cells, indicating that the changes we observed in vivo likely occur, at least in part, in the gonadotropes.

It is unusual to report that T increases just Smad2 phosphorylation and not Smad3. Ordinarily, a stimulus (activin or TGFβ) acting through the type I receptors will increase phosphorylation of both Smad2 and Smad3. One explanation for our observation of an increase in pSmad2, but not pSmad3, may be pSmad3 Ab sensitivity. As noted earlier, it was difficult to detect basal pSmad3 protein in either pituitary or αT3 cell lysates. Because we are measuring relatively small increases in Smad phosphorylation, compared with Smad activation by activin, it is possible that our assay is not sensitive enough to detect Smad3 phosphorylation in response to T. Alternatively, T may increase Smad2 phosphorylation through some unknown mechanism. There are several reports of differential activation of Smad2 and -3 in both hepatic and renal cells, which may be dependent on cell cycle stage, intracellular, and/or extracellular matrix environment and may also be independent of TGFβ or activin signaling (31, 33, 37, 46). There also appears to be differential actions of Smad2 and -3 on FSHβ transcription. Both Bernard (4) and Suszko et al. (43) report that abrogation of Smad2 or -3, by RNAi, reduces both basal and activin-induced FSHβ promoter activity, but only depletion of Smad3 reduces the magnitude increase in FSHβ response to activin. Both authors interpret these findings to indicate that, although Smad2 may not be as important in activin-induced FSHβ transcription, it does play a role in maintaining basal FSHβ levels. Additionally, Lamba et al. (29) found that overexpression of Smad2 in combination with Smad4 increased FSHβ promoter-reporter activity in LβT2 cells and that a combination of all three Smads (Smad2, -3, and -4) acted synergistically to increase FSHβ promoter activity fivefold greater than Smad3 and -4 alone. They also identified Smad2 in the transcriptional complex as a heterotrimer of Smad2, -3, and -4, bound to the SBE of the mouse FSHβ promoter, and hypothesized that the trimers containing Smad2, -3, and -4 may either recruit more diverse coactivators or enhance the affinity of these regulators to the FSHβ promoter when Smad2 is present in the complex (29). Therefore, T-induced Smad2 phosphorylation may increase FSHβ transcription, in part, by either augmenting basal transcription and/or acting synergistically with pSmad3 and Smad4.

It is known that Smad binding to the activin-responsive region of the FSHβ promoter may not be enough to stimulate transcription of the gene. Transcription factors such the bicoid-related homeodomain factor Pitx2 or the TALE homeodomain proteins Pbx1 and Prep1 have been reported to be important partners with the Smads in stimulating FSHβ promoter activity (2, 44). It is also possible that androgen receptor (AR) might partner with the Smads in regulating FSHβ transcription. In prostate cell lines, AR has been reported (12, 23, 27) to form protein-protein interactions with either Smad3 or Smad4 and to modulate either AR interacting with androgen-responsive DNA elements or Smads interacting with Smad-responsive regions of DNA. Of note, the activin-responsive region of the rodent FSHβ promoter that contains the SBE (−266/−269 bp; 29, 43, 44) is within a larger hormone response element (−274/−260 bp) that confers both androgen and progesterone sensitivity on the FSHβ promoter (34, 35, 45, 47). It remains to be seen whether AR, or other steroid receptors, acting through protein-protein interactions, can be part of and/or regulate the transcriptional complex that binds to the activin response region of the FSHβ promoter.

We also examined the effects of estrogen on FSHβ transcription in female rats. In female rats, E2 had no effect on FSHβ transcription, which contrasts with previous results, where E2 markedly suppressed FSHβ PT in male rats (10). The differences between the two studies may be due to sex differences but may also reflect estrogen dose. The current study used a physiological proestrus amount (~50 pg/ml), whereas in the prior study in male rats circulating E2 levels were supraphysiological at ~120 pg/ml. The effects of E2 on FSHβ transcription in vivo are likely indirect, since E2 does not stimulate FSHβ mRNA synthesis in female rat pituitary fragments or a murine FSHβ promoter-reporter construct in LβT2 cells (40, 45). E2 has been shown to decrease activin βB mRNA and increase pituitary FS mRNA and PT in female rats (15, 26, 39). Surprisingly, we found that E2 had a biphasic effect on both activin βB and FS mRNAs, increasing βB mRNA at 3 and 8 h and initially suppressing FS at 3 h before rebounding to 150% of controls by 24 h. Coincident with the increase in βB and decline in FS mRNAs was a sustained increase in Smad3 mRNA and transient increases in Smad2 and -7 mRNAs. Similar to males, Smad3 phosphorylation was extremely low and was unchanged after E2. In contrast, pSmad2 and tSmad2/3 were increased after E2. As E2 did not alter FSHβ transcription, this suggests either that the actions on activin, FS, and Smad2 and -3 (all of which could increase FSH) may be balanced by stimulation of the inhibitory Smad7. Alternatively, the changes in Smad mRNAs and phosphorylation may be occurring in other pituitary cell types, as estrogen is known to act through the TGFβ pathway to modulate lactotrope growth and differentiation (17).

In conclusion, androgens rapidly increase FSHβ PT in both male and female rats. The effects of T on FSHβ transcription are androgen specific, as they can be reproduced by DHT but not E2, and are correlated with modest, but significant, changes in the activin-signaling system. However, although changes in
Smad mRNAs and protein phosphorylation may be part of and/or facilitate androgen action of FSHβ transcription, they are not required.

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