Regulation of gonadotropin subunit gene transcription

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Abstract

Reproductive function in mammals is regulated by the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are secreted by the gonadotrope cell and act on the gonad in a sequential and synergistic manner to initiate sexual maturation and maintain cyclic reproductive function. The synthesis and secretion of LH and FSH are regulated mainly by the pulsatile release of the hypothalamic decapeptide hormone gonadotropin-releasing hormone (GnRH). The control of differential LH and FSH synthesis and secretion is complex and involves the interplay between the gonads, hypothalamus and pituitary. In this review, the transcriptional regulation of the gonadotropin subunit genes is discussed in a physiologic setting, and we aimed to examine the mechanisms that drive those changes.

Introduction

Dynamic regulation of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is essential for mammalian reproduction. LH and FSH are comprised of two glycoprotein subunits, α (common to both), and LHβ and FSHβ, which are coded by three genes located on separate chromosomes (Chin 1987, Gharib et al. 1990b). LH and FSH are secreted by the pituitary gonadotropes and act on the gonad in a sequential and synergistic manner to initiate sexual maturation and maintain cyclic reproductive function (Bäckström et al. 1982, Marshall & Kelch 1986, Wu et al. 1990). The synthesis and secretion of the gonadotropins are regulated primarily by the hypothalamic decapeptide gonadotropin-releasing hormone (GnRH), which is secreted into the hypophyscal-portal circulation in a pulsatile manner (Clarke & Cummins 1982, Levine & Ramirez 1982). The control of LH and FSH synthesis and secretion is complex and involves interplay between the gonads, pituitary and hypothalamus. LH and FSH act on the ovaries and the testes to regulate folliculogenesis, ovulation, spermatogenesis and steroidogenesis. Gonadal steroids and peptides, in turn, act at the hypothalamus and/or pituitary to regulate either positively or negatively LH and FSH synthesis and secretion. The aims of this review are to present the transcriptional regulation of the gonadotropin subunit genes in a physiologic setting and examine the mechanisms that drive those changes.

Physiologic changes in subunit gene transcription

Estrous cycle

Changes in gonadotropin subunit mRNA expression have been determined over the course of the estrous cycle in rats and mice. In rats, we have shown that LHβ and FSHβ mRNAs increase during the time of preovulatory gonadotropin surge. The increase in LHβ mRNA precedes that of FSHβ; LHβ mRNA began increasing around 1400 h, before the beginning of the LH surge; was maximal at 1700 h; and had returned to basal by
2200 h (Zmeili et al. 1986, Kerrigan et al. 1995). FSHβ mRNA did not increase until about 2200 h, 2 h after the increase in serum FSH, and was maximal at 0200 h during estrus and had returned to basal by 0800 h (Ortolano et al. 1988). In contrast, α mRNA did not change during the preovulatory surge (Zmeili et al. 1986). The periovulatory changes in the β-subunit mRNAs are coincident with increased GnRH secretion (Levine & Ramirez 1982) and result from increased gene transcription. β-subunit mRNA synthesis, as measured by nuclear run-on assays, was found to be greatest during proestrus for LHβ, and during late proestrus and estrus for FSHβ (Shupnik et al. 1989a). Similarly, FSHβ promoter activity was increased on estrus (versus metestrus) in transgenic mice harboring an ovine FSHβ promoter-luciferase (LUC) reporter gene (Huang et al. 2001a,b). In addition to the changes in subunit mRNAs that occur around the preovulatory gonadotropin surge, FSHβ mRNA expression increased at metestrus, and both α and LHβ mRNAs increased in parallel during diestrus, periods when serum LH and FSH levels are low. The mechanism(s) for the these changes are not well understood, but probably reflect the sensitivity of the subunit genes to differences in GnRH pulse frequencies and steroid milieu; and for FSHβ, changes in serum and/or intrapituitary inhibin, activin or follistatin (FS).

**Gonadectomy**

Several studies have shown that gonadotropin subunit gene expression is differentially regulated after gonadectomy. The loss of negative feedback by sex steroids at the hypothalamus results in increased GnRH pulse amplitude and frequency (Levine & Ramirez 1982). This increase in GnRH drives expression of all three subunit genes, but there are significant differences in the magnitudes of change and timing, both among the subunits and between the sexes.

In male rats, castration (CAST) results in a rapid increase in serum LH, reflecting an increase in GnRH secretion. Coincident with the increase in GnRH, all three subunit mRNAs are elevated 24 h after CAST (Papavasiliou et al. 1986, Dalkin et al. 2001, Burger et al. 2004). Whereas α and LHβ mRNA concentrations continue to increase steadily after CAST (Papavasiliou et al. 1986, Dalkin et al. 1990), increases in FSHβ mRNA are more modest and begin to decline by day 7 (Gharib et al. 1987, Dalkin et al. 1990).

The increases in subunit mRNA after CAST are regulated in part at the level of transcription. We have recently investigated the changes in subunit gene transcription after CAST by measuring subunit primary transcript (PT) concentrations (Fig. 1). These transcripts are newly formed RNA that include both exon and intron sequences, prior to RNA splicing, and thus are closely linked to gene transcription and mRNA formation (Dalkin et al. 2001). After CAST, LHβ PT increases within 8 h and remains elevated (Dalkin et al. 2001, Burger et al. 2004). Similarly, LHβ promoter activity has been reported to increase after CAST in transgenic mice harboring either a rat LHβ promoter-LUC construct (Fallest et al. 1995) or an ovine LHβ-chloramphenicol acetyltransferase (CAT) reporter (McNeilly et al. 1996). Although FSHβ mRNA was elevated within 24 h of CAST, we did not observe an acute increase in FSHβ PT (Burger et al. 2004). However, FSHβ transcription was increased 5–7 days after CAST, as measured by either nuclear run-on assays (Paul et al. 1990), PT (Dalkin et al. 2001) or FSHβ promoter activity in transgenic mice carrying an ovine FSHβ-LUC reporter gene (Huang et al. 2001a,b). In contrast, changes in endogenous α gene transcription after CAST are not consistent; we have reported that α PT was unchanged at day 7 (Dalkin et al. 2001), whereas α mRNA synthesis was increased on day 5 (Paul et al. 1990). Additionally, α promoter activity increased after CAST in transgenic mice carrying either a human or bovine α promoter-CAT reporter (Clay et al. 1993); in vitro, the activity of a human α promoter-LUC reporter was greater when transfected into pituitary cells from male rats that had been CAST for 7–21 days (versus intact male rats) (Colin et al. 1996).

The utilization of GnRH antagonists or androgens to suppress endogenous GnRH demonstrates that the increased subunit transcription after CAST is GnRH dependent. The synthesis rates of all three subunit mRNAs were no different between intact rats and 4-day CAST rats treated with a GnRH antagonist (Paul et al. 1990), and we have also shown that a GnRH antagonist suppresses both LHβ and FSHβ PT below intact levels within hours after CAST (Fig. 1) (Burger et al. 2004). Similarly, in transgenic mice, either GnRH...
antagonist or testosterone suppressed the post-
CAST increases in rat LHβ-LUC (Fallest et al. 1995), ovine LHβ-CAT (McNeilly et al. 1996), and both human and bovine α-CAT promoter constructs (Clay et al. 1993). The elevated transcription rates seen after CAST require sustained GnRH input. Administration of a GnRH antagonist to 7-day CAST rats rapidly reduced LH and FSH PT, with half-disappearance times of 2·7 and 0·75 h respectively (Fig. 2) (Dalkin et al. 2001).

Gonadotropin subunit gene expression is also differentially regulated after ovariectomy (OVX). In female rats, serum LH rises more slowly after OVX and is not significantly elevated until days 2 or 3 (Dalkin et al. 1993, Burger et al. 2001). Coincident with the increase in GnRH, both α and LHβ mRNA expression begins to rise about day 3, and continues to rise through day 21 (Dalkin et al. 1990, 1993, Burger et al. 2001). In contrast, both serum FSH and FSHβ mRNA increase rapidly after OVX; serum FSH doubles by 8 h and FSHβ mRNA by 30–60 min (Dalkin et al. 1993), and they continue to rise through day 7 (Dalkin et al. 1990, 1993, Burger et al. 2001). As in males, increased subunit mRNA levels result from elevated transcription rates; mRNA synthesis rates were increased for all three subunits in 30-day OVX rats (Shupnik et al. 1988). Recently, we have examined the changes in subunit transcription in the 7 days after OVX during the dynamic period after the loss of gonadal feedback (Fig. 3) (Burger et al. 2001). We found that LHβ PT concentrations were increased after day 3, paralleling changes in serum LH and LHβ mRNA. In contrast, there was a biphasic change in serum FSH, FSHβ mRNA and FSHβ PT after OVX, with an acute increase at 12–24 h, followed by an additional increase after 72 h. Although α mRNA was elevated after OVX, as in our earlier studies in males, α-PT did not change.

As in males, increased GnRH secretion after OVX is an important regulator of subunit
transcription. Suppression/blockade of GnRH by administration of either a GnRH antagonist or estradiol (E₂), in vivo, reduced α and LHβ mRNA synthesis rates significantly, but had either no effect or a modest suppression on FSHβ in long-term OVX rats (Shupnik et al. 1988, 1990, Fallest & Shupnik 1994). Similarly, we found that GnRH blockade prevented the post-OVX increase in LHβ-PT, but had no effect on the acute (12–24-h) increase in FSHβ-PT and only partially suppressed the increase in FSHβ PT after day 3 (Fig. 4). Sustained GnRH secretion is also required to maintain post-OVX increases in β-subunit transcription; giving a single dose of GnRH antagonist to 7-day OVX rats rapidly reduced both LHβ and FSHβ PT, with half-disappearance times of 13 and 17 min respectively (Fig. 5).

While α and LHβ transcription after OVX appear to be largely regulated by GnRH, control of FSHβ transcription is more complex. The modest effect of either GnRH antagonist or E₂ in suppressing FSHβ transcription in vivo, hinted at a role for inhibin, activin and/or FSH. We previously showed that administration of inhibin-α antisera to intact female rats mimics the GnRH-independent increases in FSH and FSHβ mRNA that occur within hours after OVX (Dalkin et al. 1993, 1998). Similarly, we found that FSHβ PT levels increased rapidly after inhibin immunoneutralization and were suppressed in OVX rats treated with recombinant human inhibin, suggesting that inhibin suppresses FSHβ transcription (Burger et al. 2001). However, we also found that inhibin suppressed FSHβ mRNA levels much faster than after a GnRH antagonist, suggesting that inhibin may also affect FSHβ mRNA stability.

Several transgenic mouse models have been used to investigate the effects of OVX on subunit promoter activity. Hamernik et al. (1992) reported that E₂ suppressed activity of either human αCAT or bovine αCAT constructs in OVX mice and that OVX levels could be restored with pulsatile GnRH. Activity of rat, ovine and bovine LHβ promoter–reporter constructs increased after OVX in transgenic mice, and were suppressed by either GnRH antagonist (Fallest et al. 1995, Quirk et al. 2001) or E₂ (Fallest et al. 1995, McNeilly et al. 1996). Huang et al. (2001a, b) reported that FSHβ promoter activity is increased after OVX in transgenic mice harboring an ovine FSHβ–LUC construct.

Figure 2 Disappearance rates for the gonadotropin subunit primary transcripts (PT) in 7-day CAST rats. PT concentration (fmol PT/100 µg RNA) is shown on the vertical axis, and time after GnRH antagonist treatment (h) is shown along the horizontal axis. The shaded areas represent the range observed in intact animals. The calculated t₁/₂ for the LHβ and FSHβ subunits is displayed on the respective curves. Reproduced with permission from Dalkin et al. 2001.
Physiologic changes in subunit transcription by pulsatile GnRH

A central question in studying the physiology of the gonadotropins is how a single hormone (GnRH) acting on a single cell type (gonadotrope) can differentially regulate two hormones (LH and FSH). The answer is that GnRH differentially regulates LH and FSH synthesis via changes in the pattern of GnRH pulse secretion. Our laboratory and others have investigated the effects of GnRH pulse amplitude and frequency on subunit mRNA expression both in vivo and in vitro. In GnRH-deficient male rats, fast-frequency GnRH pulses (every 8 min) favored expression of α and, to a lesser extent, LHβ mRNA, fast-physiologic GnRH pulses (every 30 min, the approximate frequency of GnRH pulses found in CAST male rats or intact female rats on the afternoon of proestrus (Levine & Duffy 1988, Levin & Ramirez 1982)) increased all three subunit mRNAs, and slow-frequency pulses (>120-min intervals) selectively increased FSHβ mRNA (Haisenleder et al. 1988, Dalkin et al. 1989, Kirk et al. 1994). GnRH pulse amplitude also differentially regulates subunit mRNA, though to a lesser extent than pulse frequency; only LHβ mRNA expression was sensitive to GnRH pulse amplitude and was maximally stimulated by GnRH pulse doses of 25 ng or less (Haisenleder et al. 1988, Iliff-Sizemore et al. 1990).

GnRH regulation of subunit mRNA is somewhat different in female rats. In initial studies, we reported that GnRH pulses increased α and FSHβ mRNA levels, but not LHβ in GnRH-deficient OVX rats (Kerrigan et al. 1993), a finding which was consistent with the lack of LHβ mRNA response to GnRH reported in cultured pituitary cells from OVX rats (Weiss et al. 1990). Subsequently, we found that testosterone was required for GnRH pulses to increase LHβ mRNA in OVX rats, and that the optimal dose of...
Testosterone was similar to levels seen at proestrus (Yasin et al. 1996). Although testosterone is required in females, it is not acutely required in males, as GnRH increased LHβ mRNA in short-term CAST males with or without T (Yasin et al. 1996). On a background of proestrus T levels, we have found

**Figure 4** The effects of GnRH antagonist on gonadotropin subunit primary transcript (PT) concentrations after OVX. Pituitaries were collected from intact rats, and 12- and 72-h OVX rats treated either with the GnRH antagonist LRF-147 (+) or vehicle (−, BSA-saline) n=5–7/group. Each bar represents the mean±S.E. * Indicates means are significantly different (P<0.05) from intact rats. ** Indicates antagonist-treated group is significantly different (P<0.05) from OVX (vehicle, −) at the same time point. Reproduced with permission from Burger et al. 2001.

**Figure 5** The effects of GnRH antagonist on β-subunit primary transcript (PT) half-disappearance times (t₁/₂). Pituitaries were collected from 7-day OVX rats treated with the GnRH antagonist LRF-147 (30 µg i.v.) and then killed 0, 30, 60 or 120 min later (n=5–6/time). Each point represents the mean±S.E. The shaded areas represent the mean ± S.E. observed in intact rats. The calculated t₁/₂ for LHβ and FSHβ primary transcripts is displayed on the respective curves. Reproduced with permission from Burger et al. 2001.
that GnRH pulse amplitude and frequency differentially regulate subunit mRNA expression in females similar to males. Alpha and FSHβ mRNAs were increased by a wider range of GnRH pulse amplitudes, while LHβ mRNA was maximally stimulated by lower GnRH pulse doses (Dalkin et al. 1999). Fast-physiologic GnRH frequencies (8–120 min intervals) stimulated all three subunit mRNAs, but only slow-frequency GnRH pulses (every 240 min) increased FSHβ (Dalkin et al. 1999). The effects of GnRH pulse amplitude and frequency have also been examined in cultured pituitary cells. In female rat pituitary cells, only α mRNA increased with high-amplitude GnRH pulses, whereas LHβ and FSHβ mRNAs responded to lower doses of GnRH (Haisenleder et al. 1993a). In male pituitary cells, α mRNA was increased by all GnRH pulse frequencies examined, LHβ mRNA was maximally stimulated by pulses every 30 min, and FSHβ mRNA was greatest after 120-min pulses (Kaiser et al. 1997b).

Although there is some evidence that GnRH may regulate subunit mRNA concentrations via mRNA stability (Salton et al. 1988, Weiss et al. 1992), GnRH appears to exert its main action at the transcriptional level. While continuous GnRH stimulated α transcription in vitro (Shupnik 1990), a pulsatile GnRH input was required to increase β-subunit transcription both in vitro (Haisenleder et al. 1991) and in vivo (Shupnik 1990, Shupnik & Fallest 1994). Furthermore, pulsatile GnRH increased β-subunit transcription in a frequency-dependent manner. Faster GnRH pulse frequencies (≥60-min interpulse interval) preferentially increased LHβ mRNA synthesis or PT levels both in vitro (Shupnik 1996) and in vivo (Haisenleder et al. 1991, Burger et al. 2002). In contrast, slower GnRH pulse frequencies (≥60-min intervals) preferentially increased FSHβ transcription. Alpha-subunit transcription does not appear to be tightly regulated by GnRH pulse frequency, some studies indicating that α transcription is favored by fast-frequency GnRH pulses (Haisenleder et al. 1991, Shupnik et al. 1996), and others showing that fast and slow frequencies are equally effective (Haisenleder et al. 2001, 2003a, Burger et al. 2002).

The effects of a GnRH pulse on subunit transcription are both rapid and transient. In male rats, LHβ and FSHβ PTs increased six- and fourfold 5 min after a GnRH pulse, and declined to basal levels by 30 min, with FSHβ PT decreasing faster than LHβ PT (Dalkin et al. 2001). Although α PT tended to increase 5–15 min after a GnRH pulse, the rise was not statistically significant. The duration of GnRH pulsatile treatment also appears to be important in regulating subunit transcription rates. In earlier studies, we found that GnRH pulses given every 30 min to male rats increased α, LHβ and FSHβ mRNA synthesis rates, but that the increases in LHβ and FSHβ returned to basal at 4–24 h (Haisenleder et al. 1991). In a more recent study, we found that only fast-frequency GnRH pulses (30 min) increased LHβ PT levels, and that the increase in transcription was sustained after 1–24 h of pulses (Fig. 6) (Burger et al. 2003).

Unexpectedly, we found that both fast- and slow-(240-min) frequency GnRH pulses increased FSHβ PT, but with significantly different time courses. Fast-frequency GnRH pulses transiently increased FSHβ PT after 1–6 h, but returned to control levels by 24 h. In contrast, slow-frequency GnRH pulses resulted in a delayed but sustained increase in FSHβ PT at 8–24 h, and only 240-min pulses increased both FSHβ PT and mRNA expression.

A recent study investigated the effects of GnRH pulse frequency on subunit promoter activities in gonadotrope-derived LβT2 cells and found similar results to those seen in vivo or in cultured pituitary cells (Bedecarrats & Kaiser 2003). Fast-frequency GnRH pulses (≥60 min) stimulated expression of a rat LHβ promoter-reporter to a greater degree than slower frequencies. In contrast, a rat FSHβ promoter-reporter was preferentially stimulated by slower GnRH pulse frequencies (≥60 min), but a clear preference for a slower GnRH frequency was not observed until after 20 h of pulses, before which fast GnRH frequencies were as effective if not better in stimulating FSHβ promoter activity (Bedecarrats & Kaiser 2003). Alpha promoter activity was also stimulated by GnRH in LβT2 cells but was less frequency dependent than the β-subunits; human α-LUC reporter activity increased with all GnRH frequencies but was greatest with more frequent GnRH pulses (Bedecarrats & Kaiser 2003).

Intracellular mechanisms of subunit gene transcription by GnRH

The GnRH receptor (GnRH-R) is a member of the seven-transmembrane receptor family, with receptor binding activating two specific mechanisms...

As discussed previously, gonadotropin subunit gene transcription is regulated in a differential manner by alterations in GnRH pulse frequency. Recent data suggest that various mammalian species express a second GnRH isoform (GnRH II) and GnRH-R (GnRH-R II) in the hypothalamus and pituitary (Neill 2002). Although effects on sexual behavior and preferential actions on FSH secretion have been described, the physiologic roles of these isoforms remain to be determined. The evidence to date suggests that differential synthesis and secretion of LH and FSH is the product of altered patterns of pulsatile GnRH I secretion by activation of pituitary GnRH-R I. Although the intracellular messengers responsible for transmitting frequency-dependent signals from the plasma membrane to the nucleus within the gonadotrope have yet to be fully characterized, recent findings have provided insights into critical sites in the signal transduction pathways involved.

**Figure 6** The effects of fast- and slow-frequency GnRH pulses over 24 h on gonadotropin subunit primary transcripts (PT). CAST plus testosterone-replaced male rats were i.v. pulsed with 25 ng GnRH every 30 (fast) or 240 (slow) min for 1–24 h (\(n=4–8\) rats/observation). Data are expressed as fold change versus controls (0 h). * Indicates significant differences (\(P<0.05\)) versus untreated, CAST plus testosterone (T) controls (0 h). ** Indicates significant differences between GnRH pulse regimens at 24 h. Reproduced with permission from Burger et al. 2002.
cAMP

Activation of the cAMP pathway stimulates $\alpha$ (mouse, rat and human) promoter activity (Maurer et al. 1999), and cAMP may also have a general stimulatory response to GnRH signaling. The mouse GnRH-R promoter contains two cAMP responsive element-binding (CREB) sites, and increased cAMP/protein kinase A (PKA) activity enhanced mouse GnRH-R promoter activity in lactotrophic GH3 cells stably transfected with rat GnRH-R cDNA (GGH3 cell lines) (Han & Conn 1999, Lin & Conn 1999). However, studies conducted in either gonadotrope-derived αT3 cells or transgenic mice models reveal that PKC, MAPK and activator protein-1 (AP-1) play a more prominent role in GnRH regulation of the GnRH promoter (Norwitz et al. 1999, White et al. 1999, Ellsworth et al. 2003). In the human model, CREB plays an essential role in GnRH activation of the $\alpha$ promoter, and GnRH stimulates the phosphorylation of CREB (Delegeane et al. 1987, Duan et al. 1999). The porcine FSH$\beta$ promoter also contains a putative CREB site (Kato et al. 1999). We have conducted in vitro studies in rat pituitary cells to determine whether cAMP stimulates gonadotropin subunit mRNA (Haisenleder et al. 1999). The results demonstrated that a diffusible cAMP analog stimulated a rise in $\alpha$, but not LH$\beta$ or FSH$\beta$ mRNA. Of interest, we showed that the pattern of cAMP input was critical, as pulsatile, but not continuous, cAMP enhanced $\alpha$ mRNA expression. More recent studies suggest that the cAMP/PKA pathway plays a role in cross-talk between specific intracellular messenger systems (that is, PKC and ERK) in response to GnRH stimulation (Garrel et al. 1997, Han & Conn 1999, Lin & Conn 1999, Fowkes et al. 2002).

Calcium

The GnRH-induced increases in intracellular calcium play an essential role in gonadotropin secretion (Naor 1990, Stojilkovic & Catt 1995a, b). Studies in various species and experimental models have also shown that $\alpha$, LH$\beta$ and FSH$\beta$ gene expression are regulated by increases in intracellular calcium (Ben Menahem & Naor 1994, Saunders et al. 1998, Weck et al. 1998). In the rat, calcium stimulates a rise in $\alpha$, LH$\beta$ and FSH$\beta$ mRNA levels (Ben Menahem & Naor 1994, Haisenleder et al. 1995b, 1997). Other reports reveal that calcium stimulates gonadotropin subunit transcriptional activation, including increases in $\alpha$ and LH$\beta$ transcription (as measured by nuclear run-on assay or promoter–luciferase construct assay) (Weck et al. 2000) and $\alpha$, LH$\beta$ and FSH$\beta$ PT (Haisenleder et al. 2001). In contrast, other investigations using GH3 cells that express GnRH-R (GGH3–1 cells) have shown that the response to calcium is selective, stimulating $\alpha$ but not LH$\beta$ and FSH$\beta$ (Saunders et al. 1998). Another report found that calcium suppresses GnRH- or PKC-induced rat LH$\beta$ promoter activity in L$\beta$T4 cells (Vasilyev et al. 2002a). The reasons for these different gonadotropin subunit responses to calcium remain to be determined. However, it is likely that factors such as differences in cell model, promoter constructs used, experimental paradigms and the end products measured play a role in the outcomes seen.

Gonadotrope cells express spikes and oscillations in intracellular calcium, the patterns of which are altered by GnRH and linked to LH secretion (Holl et al. 1988, Tse & Hille 1992, Stojilkovic & Catt 1995b). These observations are of interest, as in other cell types, alterations in intracellular calcium can play a role in the regulation of gene expression and activation of downstream mediators of signal transduction pathways (that is, ERK and calcium/calmodulin-dependent kinase II (Ca/CaMK II)) (Villalobos et al. 1988, Gu & Spitzer 1995, Dolmetsch et al. 1997, De Koninck et al. 1998, Durham & Russo 2000). We have investigated whether alterations in intracellular calcium are involved in transmission of frequency-dependent signals from the plasma membrane to the nucleus, and whether these signals mediate the $\alpha$, LH$\beta$ and FSH$\beta$ transcriptional responses to pulsatile GnRH (Haisenleder et al. 2001). The model used cultured rat pituitary cells and pulses of the calcium channel activator, Bay K 8644, plus potassium chloride (BK+KCl) given at intervals of 15, 60 or 180 min for 6 h. Gonadotropin subunit PT was measured to determine transcriptional responses to treatment. BK+KCl pulses stimulated release of both LH and FSH with a similar pattern and magnitude to those seen after pulsatile GnRH. Alterations in the frequency of calcium pulse signals revealed that $\alpha$ was selectively stimulated by faster (every 15 min) pulses of BK+KCl, with slower pulses being ineffective (Fig. 7). LH$\beta$ PT was stimulated by 15- or 60-min pulses, but not 180-min pulses, of
BK+KCl. In contrast, FSHβ PT was maximally stimulated by the slower (180-min) pulse interval. This pattern of divergent gonadotropin subunit responses to pulse frequency is similar to previous findings for GnRH (Haisenleder et al. 1997). Thus, intermittent changes in intracellular calcium appear to be important in the transmission of GnRH signals, and may mediate the differential actions of pulse frequency on gonadotropin subunit gene expression.

The downstream mediators of calcium action within the gonadotrope have yet to be characterized. Among the putative candidates is Ca/CaMK II, which is an important mediator of calcium signaling in several cell types, including the pituitary (Miller & Kennedy 1986, Hanson & Schulman 1992, Cui et al. 1994, 1996, Schulman et al. 1995, Nowakowski et al. 1997). We have examined this issue in both primary pituitary and LβT2 cells (Haisenleder et al. 2003a,b). GnRH stimulated Ca/CaMK II activity, and activation was mediated via increases in intracellular calcium derived from cellular storage pools and influx from plasma membrane calcium channels. Moreover, inhibition of Ca/CaMK II activation by administering a specific blocker, KN-93, partially suppressed GnRH-induced increases in α, LHβ and FSHβ PT (Fig. 8) or rat α and LHβ promoter activity. These finding support the hypothesis that Ca/CaMK II is a downstream mediator of GnRH/calcium signaling within the gonadotrope.

Protein kinase C (PKC)

Several investigations have shown that GnRH activates PKC, and that cross-talk between PKC, calcium and other intracellular pathways regulates gonadotropin secretion and mRNA expression (Naor 1990, Stojilkovic & Catt 1995b, Kaiser et al. 1997a, Ando et al. 2001). Studies in rat pituitary cells reveal that PKC stimulates a rise α, LHβ and FSHβ mRNAs and α transcription (Ben Menahem & Naor 1994, Weck et al. 1998, Halvorson et al. 1999, Vasilyev et al. 2002a). We have shown that PKC stimulates α and LHβ gene expression in rat pituitary cells in vitro; however, unlike results seen for cAMP and calcium, a pulsatile signal pattern does not play a significant role (Haisenleder et al. 1995). Data from other investigations in gonadotrope-derived LβT2, LβT4 or αT3 cells, or lactotrope-derived GGH3–1 cells, demonstrate that PKC mediates the α, LHβ and FSHβ transcriptional response to GnRH (Sundaresan et al. 1996, Weck et al. 1998, Vasilyev et al. 2002a,b). Kaiser

Figure 7 Effect of the calcium channel activator Bay K 8644 (BK) pulse interval (15, 60 or 180 min) on LHβ, and FSHβ primary transcript (PT) levels. Pulse treatments were given for 6 h, and a GnRH pulse group (60-min interval) was included for comparison. Results are expressed as the percent change versus vehicle-pulsed controls (Con). Means±S.E.M. are shown; note the different scale for each panel. The number of chambers per group was 10–12, derived from four or five separate experiments. *P<0.05 versus control, *P<0.05 versus 15 pulse BK group; +P<0.05 versus peak BK treatment group. Reproduced with permission from Haisenleder et al. 2001.
et al. (2000) reported that PKC stimulation of the rat LHB gene is mediated via actions on Egr-1 binding within the proximal GnRH responsive region of the LHB promoter. An investigation of the bovine LHB promoter showed that interactions between Egr-1, SF-1 and Ptx-1 mediate the response to PKC (Tremblay & Drouin 1999). PKC has also been shown to stimulate the expression of the Egr-1 gene (Wolfe & Call 1999). In contrast, α-subunit transcriptional responses to PKC reflect activation of the MAPK pathway (as discussed below). Characterization of the ovine FSHβ promoter showed that PKC activation is mediated through multiple responsive regions, including 2 AP-1 sites (Strahl et al. 1998). However, these sites do not appear to play an important role in regulating the ovine FSHβ promoter in transgenic mice (Huang et al. 2001b). Of interest, responses of individual gonadotropin subunits to PKC stimulation have been shown to differ between published reports, possibly reflecting differences in cell model or experimental paradigm (Saunders et al. 1998, Weck et al. 1998, Vasilyev et al. 2002a,b).

Mitogen-activated protein kinase (MAPK)


We conducted studies to determine whether ERK plays a role in the differential gonadotrope responses to physiologic GnRH stimulation (Haisenleder et al. 1998). Initial work examined whether a pulsatile signal pattern is required to maintain ERK responsiveness to GnRH over longer durations. Groups of adult GnRH-deficient rats were given pulses of GnRH every 60 min for 1–8 h in vivo, and were compared with animals that received a continuous GnRH infusion for the same duration. Pulsatile GnRH stimulated a two- to fourfold increase in ERK activity, which was maintained over the 8-h experimental duration. However, continuous GnRH stimulated a transient rise in ERK only for the initial 2 h (Fig. 9). Follow-up studies showed that ERK activity is sensitive to GnRH pulse frequency, with slower

![Figure 8](https://www.endocrinology-journals.org)
also maximally stimulated by slower GnRH pulse intervals (FSHβ and GnRH-R).

GnRH activation of the JNK pathway is mediated through PKC activation of the protein tyrosine kinase – cSrc (Levi et al. 1998). Recent findings suggest that JNK plays a role in the regulation of the rat LHB promoter, via actions on the proximal GnRH-responsive region of the gene (Yokoi et al. 2000). The p38 pathway has also been implicated in the activation of various transcription factors, including c-Jun, c-Fos and Elk-1 (Ham et al. 1997, Whitmarsh et al. 1997, Roberson et al. 1999). Experiments using αT3 cells revealed that PKC mediates the GnRH-induced activation of p38 (Roberson et al. 1999). However, while p38 activates downstream pathways known to stimulate α gene expression (Maurer et al. 1999), published reports suggest that the signal transduction protein does not stimulate α promoter activity (Roberson et al. 1999). Whether p38 regulates the expression of the LHB and FSHβ genes remains to be determined.

In summary, present information suggests that differential regulation of gonadotropin subunit gene expression by GnRH involves several mechanisms. GnRH may exert selective actions on specific signal transduction pathways (such as ERK regulation of FSHβ and GnRH-R, but not LHβ). Alternatively, the responses may reflect differential sensitivity to intracellular messenger systems (for example, specific gonadotropin subunit genes may be more sensitive to calcium or PKC pathways than other subunit genes). Furthermore, it is likely that the role played by GnRH pulse frequency is selectively to activate one or more intracellular signal transduction pathways that are optimal for a specific gonadotropin subunit gene.

**Regulation of subunit gene transcription by steroids**

Steroids regulate gonadotropin subunit gene expression by acting either at the hypothalamus to alter GnRH pulsatility or directly on the pituitary gonadotropes. Differential regulation of the subunit genes by steroids was first reported in gonadectomized (GDX) animals. Both E2 and testosterone suppressed the post-GDX increases in pituitary α and LHβ mRNAs. In contrast, the post-OVX increase in FSHβ mRNA was only...
partially reduced by E$_2$ or E$_2$ plus progesterone (P$_4$), reflecting the importance of ovarian inhibin in suppressing FSHβ expression (Gharib et al. 1987, Dalkin et al. 1990, 1993); in CAST rats, testosterone either had no effect or enhanced FSHβ responses in a dose-dependent manner (Gharib et al. 1987, 1990a, Wierman et al. 1988, 1990, Iliff-Sizemore et al. 1990). Experiments in GDX plus GnRH antagonist-treated rats, to eliminate the effects of steroids on GnRH secretion, largely show that in vivo steroids inhibit both α and LHβ gene expression by suppressing GnRH. In contrast, the regulation of FSHβ by steroids appears to be more complex.

α-subunit

In vivo, E$_2$ markedly suppresses α-subunit gene transcription. It inhibited the post-OVX increase in α-subunit mRNA synthesis (Shupnik et al. 1988), and suppressed the post-OVX or -CAST increases in α promoter activity in transgenic mice harboring either a human or bovine α promoter-reporter construct (Keri et al. 1991, Hamernik et al. 1992,
Clay et al. 1993). However, there is evidence that the suppression of α subunit transcription by E₂ is largely indirect. Shupnik et al. determined that E₂ reduces α transcription in vivo largely by suppressing hypothalamic GnRH; E₂ had no effect on α-subunit mRNA synthesis in rat pituitary cells in vitro (Shupnik et al. 1989a, Shupnik 1996) or in OVX rats treated with GnRH antagonist (Shupnik & Fallest 1994). Additionally, a functional estrogen response element (ERE) in the human α promoter has not been identified (Keri et al. 1991), and E₂ does not affect human α promoter-reporter activity in αT3 cells, even in the presence of an exogenous human estrogen-receptor (ER) (Clay et al. 1993). Although it appears that E₂ regulates α transcription largely via hypothalamic GnRH, it may also have a direct effect on the pituitary. Colin et al. (1996) reported that E₂ suppressed basal activity of a human α-promoter-LUC construct in the pituitaries of OVX transgenic mice, but enhanced GnRH responsiveness. We and others have also observed that E₂ potentiates GnRH stimulation of α gene expression, but it is unknown whether the effect is direct or indirect (that is, increasing GnRH receptor numbers) (Mercer et al. 1989, Dalkin et al. 1990, Kerrigan et al. 1993, Turgeon et al. 1996, Kawakami & Winters 1999).

In vivo, progesterone (P₄) potentiates the suppressive effects of E₂ on α-subunit mRNA, largely via decreased GnRH secretion (Simard et al. 1988, Dalkin et al. 1990). The effects of P₄ directly on the pituitary are mixed; P₄ (+E₂) reduces α mRNA in OVX, hypothalamically disconnected ewes (Di Gregorio & Nett 1995), and either reduces (Dalkin et al. 1990) or has no effect in the rat (Kerrigan et al. 1993). Little is known about P₄ actions on α-subunit transcription; however, neither P₄ nor the P₄ receptor antagonist RU486 modulated E₂ ± GnRH-induced changes in human α-LUC activity in pituitary cells from transgenic mice (Colin et al. 1996).

Androgens, like estrogens, suppress α subunit expression either by reducing hypothalamic GnRH or by direct action on the pituitary. In vivo, testosterone suppressed α mRNA and mRNA synthesis rates in male rats even in the presence of GnRH antagonist (Paul et al. 1990). Similarly, testosterone or dihydrotestosterone (DHT) suppressed the post-CAST increases in α promoter activity in transgenic mice harboring either a human or bovine α promoter-CAT reporter gene (Clay et al. 1993). The human α-CAT reporter gene was also repressed by DHT when transiently transfected into αT3 cells along with androgen receptor (AR), with suppression requiring both AR and ligand (Clay et al. 1993). A putative androgen response element (ARE) has been identified in the proximal promoter of the human α gene between tandem cAMP response elements (CRE) and a CCAAT element (Clay et al. 1993). However, this binding site does not mediate the suppressive effect of androgen on α transcription; instead, AR interferes with the proteins that bind an upstream α-basal element and the CRE (Heckert et al. 1997). Specifically, AR suppresses α promoter activity by protein–protein interactions with the two CRE-binding transcription factors cJun and ATF2 (Jorgensen & Nilson 2001a).

The effects of glucocorticoids on α gene expression are inconclusive. In vivo, corticosterone (B) either increases (Ringstrom et al. 1991), decreases (Kilen et al. 1996), or had no effect (McAndrews et al. 1994) on α subunit mRNA in rats. In pituitary-derived cell lines, dexamethasone suppressed α-subunit mRNA in αT3 cells (Akerblom et al. 1990), had no effect on α mRNA in LβT2 cells (Turgeon et al. 1996) or increased α promoter activity in GH3 cells transiently transfected with a human α-CAT construct (Gurr & Kourides 1989).

**LHβ**

Estrogens rapidly suppress the post-OVX increases in LHβ mRNA synthesis (Shupnik et al. 1988). This reflects a hypothalamic action, as the post-OVX increases in LHβ transcription were abolished by a GnRH antagonist (Dalkin et al. 1993, Shupnik & Fallest 1994, Fallest et al. 1995, Burger et al. 2001), and additional treatment with E₂ was no more effective than antagonist only (Shupnik & Fallest 1994). However, estrogens also exert direct action on the pituitary, and E₂ rapidly increased LHβ mRNA synthesis rates in pituitary cells from OVX female rats (Shupnik et al. 1989a, Shupnik 1996), and in pituitary cells from cycling rats, with the greatest effect in cells from rats in proestrus (Shupnik et al. 1989a). Shupnik and coworkers found that ER bound the rat LHβ promoter at −1388 to −1105 bp, an area containing a 15 bp imperfect ERE; moreover, promoter-reporter constructs of the rat LHβ gene containing the 284 bp
estrogen-responsive region, transiently transfected into GH3 cells, were estrogen responsive in an ER-dependent manner (Shupnik et al. 1989b, Shupnik & Rosenzweig 1991). However, the estrogen responsiveness of the LHβ promoter may not be conserved across the species. While E2 suppressed LHβ promoter activity in OVX transgenic mice harboring a −776 to +10 bp bovine LHβ promoter CAT reporter, a high-affinity binding site for the ER was not found in this portion of the promoter (Keri et al. 1994). In addition, E2 had no effect on bovine LHβ CAT activity when transiently transfected into LBβT2 cells, even when cotransfected with the human ER (Jorgensen & Nilson 2001b). It remains to be determined whether an estrogen-responsive region of the bovine LHβ promoter may be more 5′ than the area previously investigated. Alternatively, the ER may modulate promoter activity by interacting with other cis-acting elements, as described for androgens on the α and LHβ promoters.

There is little information on the regulation of LHβ gene expression by either P4 or glucocorticoids. Progesterone alone had no effect on LHβ mRNA levels in OVX rats and when combined with E2 was no more effective than E2 alone (Dalkin et al. 1990, Kerrigan et al. 1993). Similarly, while E2 blocked bovine LHβ CAT promoter activity in OVX transgenic mice, the combination of E2 and P4 was no more effective than E2 alone (McNeilly et al. 1996). In vitro, neither P4 nor E2+P4 altered LHβ mRNA levels in female rat pituitary cells, but P4 alone or in combination with E2 augmented the GnRH-induced increase in LHβ mRNA, perhaps by blocking LHβ mRNA degradation (Park et al. 1996). Glucocorticoids do not appear to regulate LHβ mRNA; B had no effect on LHβ mRNA levels in cultured rat pituitary cells (Kilen et al. 1996) and dexamethasone did not alter the activity of the rat LHβ promoter (Shupnik & Rosenzweig 1991).

Androgens rapidly suppress the post-CAST rise in LHβ transcription. Testosterone inhibited LHβ mRNA synthesis in CAST rats (Paul et al. 1990) and suppressed the post-CAST increases in LHβ promoter activity in transgenic mice (Keri et al. 1994, Fallet et al. 1995). We examined the effects of testosterone on LHβ transcription in vivo; testosterone rapidly suppressed LHβ PT below basal values in GnRH-deficient CAST rats (Fig. 11) (Burger et al. 2004). The suppression of LHβ PT in GnRH-deficient rats in vivo suggests that testosterone acts directly at the pituitary, but we found no effect of testosterone on LHβ PT in cultured rat pituitary cells (Burger et al. 2004). Similarly, DHT had little effect on rat LHβ promoter LUC reporter activity in LBβT2 cells, but did suppress GnRH-induced LHβ promoter activity (Curtin et al. 2001). This action required the AR even though a high-affinity binding site for the AR was not found in this construct. Curtin et al. (2001) determined that the AR suppressed rat LHβ promoter activity by protein–protein interactions with the specificity protein-1 (Sp1) transcription factor, and, to a lesser extent, with the early growth response protein (Erg-1), which are required for GnRH stimulation of LHβ transcription (Kaiser et al. 2000, Weck et al. 2000). In contrast to the rat, the bovine LHβ promoter was suppressed by testosterone (Jorgensen & Nilson 2001b). The AR, in a ligand-dependent manner, directly responsive to testosterone (Jorgenson & Nilson 2001b). The AR in the hypothalamus, as E2 did not suppress LHβ mRNA synthesis in female rat pituitary fragments (Shupnik & Fallet 1994). In contrast to the rat, E2 suppressed both steady-state FSHβ mRNA and mRNA synthesis rates in cultured ovine pituitary cells (Phillips et al. 1988, Baratta et al. 2001), and suppressed the activity of an ovine FSHβ promoter–LUC construct transfected into ovine pituitary cells (Miller & Miller 1996). The estrogen-responsive region of the ovine FSHβ promoter is located at −105 to −84 bp, but does not contain an estrogen-response element or bind ER (Miller & Miller 1996). Instead, this estrogen-responsive region contains two AP-1 sites.
and is a GnRH enhancer region of the ovine FSHβ promoter (Strahl et al. 1998). Although E2 inhibits FSHβ transcription in sheep, it had no effect on an ovine FSHβ promoter-LUC construct in female transgenic mice, and the authors suggest that mouse gonadotropes lack some factor making them less responsive to negative feedback by estrogen (Huang et al. 2001a). We recently investigated the effects of steroids on FSHβ transcription in male rats. Contrary to previous results in females, we found that E2 markedly suppressed FSHβ PT, but not FSHβ mRNA, and this may suggest that E2 differentially regulates FSHβ transcription between the sexes in rodents (Burger et al. 2004).

FSHβ transcription is also regulated by P4, and, like estrogens, there appear to be differences between species. Progesterone, in combination with E2, increased FSHβ mRNA expression in immature rats (Attardi et al. 1990) and increased the stimulatory effects of GnRH on FSHβ mRNA (versus E2 alone) (Kerrigan et al. 1993). Moreover, the preovulatory FSH surge and the accompanying increase in FSHβ mRNA can be suppressed by antiprogestins (Ringstrom et al. 1997). Three progesterone-response element (PRE)-like sequences have been identified in the proximal promoter of the rat FSHβ gene; these sites bind P4 receptor (PR) and render a heterologous promoter–reporter construct P4 responsive (O’Connor et al. 1997, 1999). In contrast, P4 suppressed endogenous FSHβ mRNA synthesis in ovine pituitary cell cultures (Phillips et al. 1988). The ovine FSHβ promoter contains six PRE-like sequences that bind PR and render a heterologous promoter reporter construct P4 responsive (Webster et al. 1995). However, contrary to the suppressive effects of P4 on endogenous FSHβ transcription, P4 stimulated an ovine FSHβ promoter-LUC reporter construct transfected into ovine pituitary cells (Webster et al. 1995).

Glucocorticoids affect FSHβ gene expression, and B selectively upregulated FSHβ mRNA levels (Ringstrom et al. 1991, McAndrews et al. 1994). These effects are at the level of the gonadotrope, as B or dexamethasone increased FSHβ mRNA in rat pituitary cells (Kilen et al. 1996, Bohnsack et al. 2000, Leal et al. 2003) and in rats treated with a GnRH antagonist (McAndrews et al. 1994). Moreover, the effects of B appear to be at the level of transcription, as it increased FSHβ mRNA in...
female rat pituitary cells, without changing post-transcriptional mRNA decay rates (Kilen et al. 1996).

The effects of testosterone on FSHβ gene expression in the rat are intriguing. As mentioned previously, testosterone does not suppress the post-GDX increase in FSHβ mRNA; instead, it either has no effect or further increases FSHβ mRNA expression (Gharib et al. 1987, 1990a, Wierman et al. 1988, 1990, Iliff-Sizemore et al. 1990). Studies in GnRH-deficient rats or in pituitary cell cultures demonstrated that the stimulatory effects of testosterone on FSHβ gene expression are at the level of the pituitary (Paul et al. 1990, Wierman & Wang 1990, Dalkin et al. 1992, Winters et al. 1992). Earlier we proposed that testosterone regulated FSHβ mRNA stability, since testosterone increased the half-disappearance time of FSHβ mRNA from 20 to 50 h, but did not significantly increase FSHβ mRNA synthesis (Paul et al. 1990). However, we recently reinvestigated the effects of testosterone on FSHβ transcription, and found that it rapidly and specifically increases FSHβ PT levels at 3–48 h in male rats (Fig. 11) and that the stimulatory effects on FSHβ transcription are androgen specific (Burger et al. 2004). Testosterone also suppresses FSH mRNA, which suggests that testosterone’s actions on FSHβ transcription may be indirect via activin/FS. Others have reported that the effects of testosterone on FSHβ mRNA either require activin (Leal et al. 2003) or are blunted by FS (Bohnsack et al. 2000). To determine whether testosterone stimulation of FSHβ PT reflected the fall in pituitary FS, we treated male rat pituitary cells with testosterone with or without exogenous FS. FS alone suppressed FSHβ PT, but did not reduce the testosterone-stimulated increase in FSHβ PT, suggesting that testosterone increases transcription by direct action on the FSHβ gene. Recently, testosterone (or DHT) was reported to increase the activity of an ovine FSHβ promoter-reporter transfected into LBT2 cells (Spady et al. 2004). Spady et al. (2004) identified three candidate androgen-response elements (AREs) at −245/−231, −212/−198 and −153/−139 bp of the ovine FSHβ promoter, which are the same steroid-binding elements identified by Webster et al. (1995) as PREs. Only the −245/−231 bp putative ARE bound the AR, although mutations in either the −245/−231 or −153/−139 ARE disrupted the stimulation of the promoter by DHT (Spady et al. 2004). In contrast to the rat, the effects of testosterone on the ovine FSHβ promoter are activin dependent. The ability of DHT to stimulate the ovine FSHβ promoter is abolished by FS treatment, is increased synergistically by activin, and requires an activin-responsive Smad-binding element (SBE) at −138/−124 bp (Spady et al. 2004). Although the AREs identified in the ovine FSHβ promoter are well conserved across mammalian species (Spady et al. 2004), the stimulatory effects of testosterone on FSHβ expression are not. Testosterone suppressed FSHβ mRNA in male rhesus pituitary cells (Kawakami et al. 2002) and a human FSHβ promoter construct in transgenic mice (Kumar et al. 1992, Kumar & Low 1993, 1995).

### Regulation of FSHβ gene transcription by activin, FS and inhibin

Differential synthesis and secretion of LH and FSH, while in part dependent on the hypothalamic GnRH signal pattern, also appears to result from the actions of multiple local and gonadal peptide hormones. The hormones inhibin, activin and FS are produced both in the gonads and, at least for activin and FS, in the pituitary gland. Alterations in the balance of activin and inhibin concentrations, either directly or via changes in FS (for activin), selectively alter FSH secretion by regulating FSHβ gene expression (Mather et al. 1997).

The inhibins and activins are members of the transforming growth factor-β (TGFβ) superfamily. The inhibins, heterodimers of an inhibin A-subunit and one of two B-subunits, βA (inhibin A) or βB (inhibin B), are produced primarily by the gonads and act in an endocrine manner to suppress pituitary FSH secretion without affecting LH (Burger et al. 1997). The activins are dimers of two inhibin β-subunits. There are at least four forms of the β subunit, βA–βD, although the βA and βB subunits are important in the regulation of FSH. The activins were discovered because of their ability to stimulate FSH synthesis and release it from pituitary cell cultures (Ling et al. 1986, Vale et al. 1986), but it is now known that the activins are produced in a wide variety of tissues and act largely in a paracrine/autocrine manner to regulate functions ranging from development to cellular homeostasis. The pituitary expresses βB, and
activin-B ($\beta_B\beta_B$) is synthesized and secreted in the pituitary, whereas most other tissues produce activin-A (Meunier et al. 1988). In addition, the activin receptors types I, IIA and IIB are also present in the rat pituitary (Dalkin et al. 1996). FS, a glycoprotein hormone structurally unrelated to the activins and inhibins, binds to and neutralizes the bioactivity of activin. The affinity of FS for activin is high, and the dissociation rate after binding is extremely slow, essentially making the binding event a permanent one (Nakamura et al. 1990). FS was discovered in ovarian follicular fluid by its ability to suppress FSH (Esch et al. 1987), but, like activin, it is also synthesized in a wide array of tissues, including the pituitary gonadotropes and folliculostellate cells (Kogawa et al. 1991a,b, Kaiser et al. 1992). Although FS can be detected in the circulation, its ability to suppress FSH is largely due to paracrine and/or autocrine regulation of bioactive activin at the level of the gonadotrope (for review, see Phillips & de Kretser 1998).

Activin is an important regulator of FSH secretion and FSH$\beta$ gene expression; it increased FSH secretion and FSH$\beta$ mRNA in a dose-dependent manner in primary rat pituitary cells (Carroll et al. 1989, Weiss et al. 1993), and increased FSH secretion in vivo (Rivier & Vale 1991, Carrol et al. 1991, Woodruff et al. 1993, Lee & Rivier 1997).

The intrapituitary regulation of activin action is important in control of FSH$\beta$ gene expression; after OVX, pituitary $\beta_B$ and ActRII mRNAs increase in parallel with FSH$\beta$ mRNA (Dalkin et al. 1994, 1998, Prendergast et al. 2004), and GnRH pulse frequencies that maximally stimulate FSH$\beta$ mRNA also stimulate $\beta_B$ mRNA (Dalkin et al. 1999). Activin increases FSH$\beta$ mRNA expression by increasing transcription. Activin increased FSH$\beta$ PT in rat pituitary cells, an effect which could be blocked by the transcription inhibitor actinomycin D (Weiss et al. 1995, Bernard 2004). Activin has also been reported to stimulate ovine, rat and mouse FSH$\beta$ promoter–reporter constructs (Huang et al. 2001b, Pernasetti et al. 2001, Dupont et al. 2003, Suszko et al. 2003, Bailey et al. 2004, Bernard 2004). In light of these data, recent studies have begun to focus on the mechanism(s) whereby activin signaling regulates promoter activity for the FSH$\beta$ gene.

Activin signaling requires a series of events, including the activation of intracellular messengers (for review, see Attisano & Wrana 1996, Pangus & Woodruff 2000, Derynck & Zhang 2003). Briefly, activin binds to its type II receptor subunit, either ActRII or IIB, which then pairs with a type I receptor subunit (either ActRI or IB), forming a heteromeric complex at the cell surface. Then serine/threonine kinase activity of the type II subunit phosphorylates the type I subunit, initiating post-receptor signaling/phosphorylation. Although a number of signal cascades may be activated as the result of activin/activin receptor association, the predominant activin-signaling pathway includes the mothers against dpp-related (Smad) proteins.

Eight Smad proteins (Smads 1–8) conduct the intracellular signals for the TGF$\beta$ super family from receptors to the nucleus (for review, see Derynck & Zhang 2003). For the activin signaling system, receptor-activated Smads2 and 3 are phosphorylated by ActRI and then partner with co-Smad4, which then binds to DNA to regulate gene activity. In pituitary cells, activin-induced increases in FSH$\beta$ transcription were correlated with increased Smad2 and 3 phosphorylation (Dupont et al. 2003, Bernhard 2004). Additionally, rat or mouse FSH$\beta$ promoter activity was increased by overexpression of Smad3 and attenuated when Smad2 or 3 protein were suppressed (Dupont et al. 2003, Suszko et al. 2003, Bernhard 2004).

The association of Smad2/3 with Smad4 and the formation of dimers is an important step in signal transduction from the cell surface (Lagna et al. 1996). Smad4 appears essential for activin signaling, as transfection of a constitutively active Smad4 construct alone can induce activin-like effects, whereas cellular expression of Smad2/3 alone does not confer responsiveness (Lagna et al. 1996), and in pituitary cells expression of Smad4 greatly enhances the ability of Smad3 to increase rat FSH$\beta$ promoter activity (Suszko et al. 2003). Additionally, Smad2/3 activity is regulated by ‘inhibitory’ Smad7, which binds the type I receptor and prevents Smad2/3 phosphorylation. Smad7 gene expression is detected in both normal pituitary cells and gonadotrope cell lines, and overexpression of Smad7 in L$\beta$T2 cells disrupted activin-induced increases in FSH$\beta$ gene expression (Dupont et al. 2003, Bernhard 2004).

To date, data suggest that there is a Smad-binding element (SBE) comprising CAGA- or GTCT-like sequences that are required, but are not sufficient, for Smad binding (Shi & Massague 2003). The affinity of Smads for SBEs is low, and
gene activation requires the presence of other cofactors. Some of these transcriptional regulators are specific to the FSHβ gene, as recent reports suggest the importance of the nuclear factor Pitx2, a member of the Pitx subfamily of bicoid-related homeodomain factors (Suszko et al. 2003). In that report, critical regions within the rat FSHβ promoter were identified for Smad3/4 binding (−281 to −253 bp) and Pitx2 (−230 to −199 bp); more importantly, both Smads and Pitx2 may physically interact in the regulation of the FSHβ promoter activation. However, the critical SBE in the rat promoter is conserved only in rodent species. In the ovine FSHβ promoter, there are three regions (−973/−962, −167 and −134) required for full activin responsiveness (Bailey et al. 2004). The distal −973/−962 site bound Smad4 protein, and the critical −134 site bound Smad4 in association with the TALE homeodomain proteins Pbx1 and Prep1, and the two proximal activin responsive regions (−167 and −134) are conserved across species and also bind Pbx1 and Prep1 in the mouse gene (Bailey et al. 2004).

Limitation of activin action appears to be an essential component of physiologic gonadotrope function. In addition to the intracellular inhibitor Smad7 described above, the extracellular inhibitors FS and inhibin play key roles in limiting activin action. In the rat pituitary, FS is upregulated by activin, GnRH and PACAP, and is suppressed by testosterone and by FS itself, probably through binding to activin (Simonaka et al. 1991, Kirk et al. 1994, Winters et al. 1997, Burger et al. 2003, 2004). Conversely, in primate pituitary cultures, GnRH is ineffective, and testosterone (as well as activin) increases FS expression (Kawakami et al. 2002). Current thoughts regarding the physiologic role of pituitary-derived FS remain speculative, as a FS-deficient cell model is lacking and knockout of the FS gene is lethal (Matzuk et al. 1995). However, it is widely regarded that pituitary FS influences the FSH response to hypothalamic and gonadal stimuli. FS expression increases after CAST in adult male rats (Kaiser & Chin 1993) and during the reproductive cycle (Halvorson et al. 1994). Additionally, there is a reciprocal relationship over time between FS and FSHβ transcription after GnRH pulses; fast-frequency GnRH pulses stimuliuated FSHβ only transiently, and this was followed by an increase in FS mRNA, whereas slow-frequency pulses suppressed FS and increased FSHβ, implying that FS attenuates the FSH response (Burger et al. 2002). In male primates, by contrast, FS mRNA is unaffected by CAST, and FSHβ mRNA increases by about 50-fold (Winters et al. 2001). Thus, FS appears to function as a brake on FSH production in rodents, but its role in other mammalian species is less certain.

Circulating inhibin is also important in antagonizing the action of activin on pituitary FSH. Serum inhibin levels in the rat are regulated across the estrous cycle and are greatest early in the cycle, peaking at proestrus and declining to their lowest levels at estrus. Low inhibin levels at estrus are coincident with increased FSH secretion, steady-state FSHβ mRNA, and FSHβ transcription associated with the secondary FSH surge (Shupnik et al. 1989a, Haisenleder et al. 1990, Woodruff et al. 1996). As mentioned previously, after OVX and the loss of circulating inhibin, there is a rapid and GnRH-independent increase in both FSHβ mRNA and FSHβ PT, which can be mimicked by giving inhibin-α antisera to intact rats (Burger et al. 2001). Inhibin administration also decreased ovine FSHβ promoter-LUC activity in pituitary cells from transgenic mice and rapidly suppressed FSHβ transcription in OVX rats and ewes (Burger et al. 2001, Huang et al. 2001a, Clarke et al. 1993). In addition to suppressing FSHβ transcription, inhibin may also regulate the stability of the FSHβ mRNA. In rats, inhibin suppressed FSHβ mRNA levels with a half-life of 94 min, much shorter than the 40-h half-life of FSHβ mRNA in vivo (Burger et al. 2001). In sheep, inhibin suppressed FSHβ transcription by only 50% but mRNA levels by 100% (Clarke et al. 1993). Suppression of FSHβ mRNA by inhibin in rat pituitary cells could also be blunted by inhibitors of translation, suggesting that inhibin stimulates transcription of a protein that reduces FSHβ mRNA stability (Attardi et al. 1991, Attardi & Winters 1993).

The mechanism for the effects of inhibin on FSHβ transcription are not clear; the inhibitors do bind the activin type II receptors, although with much lower affinity than activin, but this binding does not promote association with the type I receptor, initiate intracellular Smad signaling, or appear to have its own signaling pathway (Mathews & Vale 1991, Attisano et al. 1992, dePaolo 1997), suggesting that inhibin may work as a functional antagonist for activin at the level of the activin receptor. However, in view of inhibin’s low affinity
for the type II receptors, this does not explain inhibin action even in the presence of excess activin. Recently, two potential receptors/coreceptors for inhibin have been identified: betaglycan and inhibin-binding protein/p120 (InhBP/p120) (Chong et al. 2000, Lewis et al. 2000). However, new data suggest that betaglycan may be of greater physiologic importance (Chapman et al. 2002). Transfection of COS-7 or HEK293 cells with an expression vector encoding the full-length InhBP/p120 cDNA (alone or in addition to either of the type II activin receptors) failed to induce specific inhibin binding. In contrast, transfection with constructs containing full-length betaglycan conferred high-affinity inhibin binding with formation of an inhibin/betaglycan/type II activin receptor complex. Furthermore, betaglycan immunoreactivity localizes with gonadotrope cells in the pituitaries of both male and female rats and is correlated with high-serum inhibin, low-serum FSH, and low-pituitary FSHβ immunoreactivity just before and after the FSH surge in cycling rats (MacConell et al. 2002, Chapman & Woodruff 2003).

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