Intra-pituitary regulation of gonadotrophs in male rodents and primates

Stephen J Winters and Joseph P Moore

Division of Endocrinology, Metabolism and Diabetes, University of Louisville, ACB-A3G11, 550 Jackson Street, Louisville, Kentucky 40202, USA

Correspondence should be addressed to Stephen J Winters; Email: sjwint01@louisville.edu

Abstract

Paracrine and autocrine regulation is well established in many organs including the gonads, but the notion of communication among pituitary cells is a relatively new concept. The FSH- β and GnRH-receptor genes are up-regulated by pituitary activin and down-regulated by pituitary follistatin, and circulating inhibin disrupts this local regulation by functioning as an endogenous competitor of the activin receptor. Activin and follistatin production by folliculostellate cells may play a central role in these responses. α -Subunit expression is maintained at high levels in the absence of GnRH through unknown mechanisms. There is evidence that the intra-pituitary regulation of FSH- β and GnRH-receptor gene expression may activate pubertal maturation in male rats. Finally, there are marked differences in follistatin expression and its regulation by GnRH and androgens in male primates and rats that appear to explain species differences in the differential secretion of FSH and LH, although the physiological significance of these differences is not yet known.

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Introduction

Gonadotropin-releasing hormone (GnRH), a decapeptide from the hypothalamus, is the proximate regulator of gonadotroph function (Stojilkovic & Catt 1995, Cheng & Leung 2000, Kakar *et al.* 2002). It is becoming increasingly evident, however, that gonadotrophs are also regulated by factors produced by gonadotrophs themselves, or by surrounding pituitary cells, that generate unique effects or influence the actions of GnRH. Table 1 lists proposed autocrine and paracrine regulators of gonadotrophs. For the most part, these substances have been identified because their receptors are expressed by gonadotrophs, or through effects they produce when added to pituitary cell cultures; the physiological role of most of these factors remains to be elucidated. Microarray analysis has identified over 200 genes in gonadotrophs that are regulated by GnRH (Kakar *et al.* 2003). In this review we will examine the intra-pituitary regulation of the gonadotropin subunit and GnRH-receptor (GnRH-R) genes since much is known about these genes, and they are essential for reproduction.

While early studies sought to identify a unique hypothalamic follicle-stimulating hormone (FSH)-releasing factor to explain instances in which FSH and luteinizing hormone (LH) secretion diverge, it is now evident that FSH secretion is selectively regulated because FSH- β gene expression is increased by activin, or decreased by follistatin or inhibin.

Moreover, these factors regulate GnRH-receptors. There is evidence that activin and follistatin are produced by gonadotrophs and by pituitary folliculostellate cells, and perhaps by somatotrophs, lactotrophs and thyrotrophs. Inhibin, a product of the testis, may also be produced in small amounts in the pituitary. There are several recent reviews describing activin, follistatin and inhibin signaling (Itoh *et al.* 2000, Pangas & Woodruff 2000, Gray *et al.* 2002). Our focus will be to integrate that information with what is known about FSH- β and GnRH-receptors, to discuss the paracrine control of the gonadotropin subunits and GnRH-receptors during pubertal development in the male rat, and to highlight differences in the regulation of these genes in male primates.

Activin, follistatin and inhibin

Separation of the pituitary from GnRH stimulation in male rats abolishes LH synthesis and secretion but allows for continued production of FSH (Sheridan *et al.* 1979, Culler & Negro-Vilar 1986, DePaolo *et al.* 1991) as well as uncombined α -subunit (Grotjan *et al.* 1984). While these experiments revealed that α -subunit and FSH- β are not entirely GnRH dependent, the initial hypothesis for a system controlling the differential regulation of the gonadotropins was derived from studies of the testis (McCullagh 1932) in which a water-soluble testicular extract prevented

Table 1 Pituitary factors proposed to regulate gonadotrophs.

Factor	Action	Cell source	Species	Reference
Activin	Increase number of cells secreting FSH, amount FSH per cell	G, S, T	Rat	Miyamoto <i>et al.</i> 1999
Adenosine	Decrease FSH secretion	All	Rat	Yu <i>et al.</i> 1998
	Decrease LH, FSH secretion			Picanco-Diniz <i>et al.</i> 1996
ATP	Increase Ca ²⁺ entry; LH secretion	All	Rat	Tomic <i>et al.</i> 1996
	Increase LH secretion			Chen <i>et al.</i> 1995
CNP	Increase LH secretion	G	Rat	McArdle <i>et al.</i> 1993
EGF	Increase GnRH binding, LH response to GnRH	G, L, S, C, T	Rat	Leblanc <i>et al.</i> 1997
Follistatin	Expression linked to attenuated FSH response to GnRH	G, FS cells	Rat	Besecke <i>et al.</i> 96
Galanin	Decreases GnRH-stimulated LH and FSH secretion	L, S, T	Rat	Todd <i>et al.</i> 1998
GnRH	Increase differentiation: gonadotrophs, thyrotrophs	Rathke's	Rat E11	Heritier & Dubois 1994
	Contributes to basal LH secretion	?	Rat/αT3	Krsmanovic <i>et al.</i> 2000
GH	Increase LH, FSH secretion	S	Mouse/rat	Tang <i>et al.</i> 1993
	Decrease FSH-β, LH-β, FSH protein response to GnRH			
NO	Increase LH secretion	G, FS cells	Rat	Ceccatelli <i>et al.</i> 1993
NPY	Increase FSH and LH secretion	L	Rat	O'Conner <i>et al.</i> 1993
	Decrease LH secretion		Rat	Bauer-Dantoin <i>et al.</i> 1993
PACAP	Increase LH and FSH secretion	G, FS cells	Rat	Culler & Paschall 1991
	Decrease FSH-β mRNA			Winters <i>et al.</i> 1997
SP	Increase LH secretion	S, T	Rat	Shamgochian & Leeman 1992
	Attenuate LH, FSH responses to GnRH		Hamster	Debeljuk & Bartke 1994
TRH	Increase gonadotroph differentiation	G	Rat E11	Heritier & Dubois 1993

G, gonadotroph; L, lactotroph; S, somatotroph; C, corticotroph; T, thyrotroph; FS cells, folliculo stellate cells; GH, growth hormone; NO, nitric oxide; NPY, neuropeptide Y; SP, substance P; TRH, thyrotropin-releasing hormone.

the formation of 'castration cells' within the anterior pituitary of orchidectomized rats. Thus began the theory that an inhibitory peptide acts as an endocrine hormone to suppress gonadotroph function. Although shrouded in controversy for many years (de Jong 1979), some 50 years later, this peptide, aptly named inhibin, was isolated from follicular fluid and demonstrated to inhibit selectively the production of FSH (De Jong 1988). Soon thereafter using similar methods, two other peptides were identified that also regulate FSH, activin (Ling *et al.* 1986, Vale *et al.* 1986) and follistatin (Robertson *et al.* 1987, Ueno *et al.* 1987) that together established an intra-pituitary mechanism for the control of gonadotrophs.

Activins are protein dimers of two β-subunits that have been designated activin-A (β_Aβ_A), activin-B (β_Bβ_B) and activin-AB (β_Aβ_B) (Vale *et al.* 1988). Activin-B appears to be produced by the pituitary since adding an antiserum to activin-B decreased FSH-β and FSH secretion (Corrigan *et al.* 1991) as well as activin/inhibin-β_B itself and follistatin mRNA concentrations in rat pituitary cultures (Bilezikjian *et al.* 1996). The β_B-subunit gene (Bilezikjian *et al.* 1996, Dalkin *et al.* 1998) and protein (Roberts *et al.* 1989) are also more abundant in the rat pituitary than is β_A, and β_B mRNA is found in the monkey pituitary where β_A mRNA is expressed faintly (Attardi *et al.* 1992). Immunohistochemical studies of the human pituitary revealed β_B in gonadotrophs and thyrotrophs, whereas β_A was localized to gonadotrophs, somatotrophs and lactotrophs (Uccella *et al.* 2000).

Inhibin is composed of an α-subunit disulfide linked to one of two β-subunits, the β_A-subunit to form inhibin-A, or the β_B-subunit to form inhibin-B (Ying 1988); inhibin-B is also the form secreted by the testis (Anawalt *et al.* 1996,

Woodruff *et al.* 1996). Inhibin α-subunit mRNA is found in the rat pituitary in low concentration using solution hybridization methods (Bilezikjian *et al.* 1996) but was undetectable by northern hybridization in monkey pituitary RNA (Attardi *et al.* 1992). However, an antiserum to porcine α-inhibin identified immunoreactive inhibin throughout the pars distalis of the crab-eating and rhesus monkey pituitary (Schlatt *et al.* 1991) suggesting that the pituitary may also produce inhibin in the male primate and rat.

Like other members of the transforming growth factor (TGF)-β superfamily of proteins, activins and inhibins initiate their actions by binding to a complex of transmembrane serine and threonine kinase receptors. Activin binds an activin type-II receptor, and then an activin type-I receptor is recruited into the complex. Once activated, the type-I receptor kinase stimulates a novel family of proteins called Smads (Attisano & Wrana 1998). Activin signaling is relayed through Smad2 and Smad3. Once activated by phosphorylation, Smad2 or Smad3 associate with the common Smad4, and the dimer migrates to the nucleus to bind specific promoters, known as Smad-binding elements (SBEs). SBEs are ubiquitous and not sufficient for activin activation, and signaling specificity for gonadotrophs presumably requires cell-specific transcriptional cofactors and co-activators (Massague & Chen 2000).

Inhibins are thought to exert their antagonistic effects by competing with activins for binding to the activin receptor II presumably through the β-subunit they share with activin (Xu *et al.* 1995). Inhibin fails, however, to stimulate phosphorylation of the type-I receptor. While inhibin at low concentrations blocks activin signaling, it binds recombinant activin receptor II with only 10% of the

affinity of activin (Mathews & Vale 1991). It is now known that betaglycan functions as an inhibin co-receptor to increase its binding affinity for activin receptor II (Gray *et al.* 2002). In this way, the endocrine hormone inhibin is present in sufficient concentration to block the effect of locally produced activins.

Follistatin, a cysteine-rich glycosylated monomeric protein that is structurally unrelated to inhibin and activin, is co-expressed with activin in essentially all tissues (Kawakami *et al.* 2001), and functions as a high-affinity activin-binding protein (Nakamura *et al.* 1990). There are two forms of follistatin mRNA generated by alternative splicing which code for follistatin-315 and its carboxy-truncated form, follistatin-288. Recombinant human follistatin-288 has 8- to 10-fold greater FSH suppressing ability than follistatin-315 (Inouye *et al.* 1991), but is a minor form representing <5-10% of follistatin-315 mRNA (Kawakami *et al.* 2001). With a short biological half-life (Kogure *et al.* 1996) follistatin is able to rapidly modulate activin binding to its receptors (Phillips & de Kretser 1998).

Follistatin appears to be expressed by all pituitary cells (Lee *et al.* 1993). Attention has focused on gonadotrophs and folliculostellate cells, however, since there is evidence that the latter cell type serves a paracrine function in the pituitary. Folliculostellate cells account for about 10% of the cells in the rat anterior pituitary. These star-shaped cells encircle well-defined cavities, and extend cytoplasmic processes (Soji *et al.* 1997) that connect with other folliculostellate cells by gap-junction channels (Fauquier *et al.* 2001) and communicate by calcium waves (Inoue *et al.* 1999).

Paracrine regulation of the gonadotropin subunit and GnRH-receptor genes

Many studies have shown that activin is the pituitary-derived factor that selectively stimulates FSH synthesis. Activin stimulates FSH secretion and FSH- β mRNA levels *in vitro* and *in vivo* (Ling *et al.* 1986, Vale *et al.* 1986, Weiss *et al.* 1993), and FSH- β mRNA and circulating FSH are reduced in mice deficient in the activin receptor (Matzuk *et al.* 1995, Kumar *et al.* 2003). When rat pituitary cell cultures are prolonged in the absence of GnRH, FSH- β mRNA expression is maintained, and a substantial amount of FSH but little LH is produced (Farnworth 1995). In this model, blocking activin receptors with inhibin, or adding follistatin to bind activin, reduces FSH- β mRNA levels and FSH secretion (Ying 1988). Furthermore, immunoneutralization with an antibody to activin-B inhibited FSH release and reduced FSH- β gene expression (Corrigan *et al.* 1991).

Activin increases FSH- β expression at the level of transcription since nuclear transcripts in rat pituitary cells are increased by activin, and the increase is blocked by actinomycin-D (Weiss *et al.* 1995). Activin stimulates the ovine FSH- β proximal promoter in pituitary cells from mice expressing the o-FSH- β transgene (Huang *et al.* 2001) and augments up-regulation by GnRH (Pernasetti

et al. 2001). Activin signaling to the FSH- β promoter is through Smad-3 and is augmented by Smad-4, and gonadotroph-specific expression of FSH- β is partly explained by Pitx2, a factor required for pituitary development, that binds the rat FSH- β promoter and stimulates basal and activin-stimulated transcription (Suszko *et al.* 2003). Activin also increases FSH- β mRNA levels by prolonging the half-life of FSH- β mRNA transcripts (Carroll *et al.* 1991, Attardi & Winters 1993).

The glycoprotein α -subunit gene is expressed by gonadotrophs and thyrotrophs, and during fetal development is one of the first indicators of pituitary cell differentiation (Rosenfeld *et al.* 2000). Rat anterior pituitary cells in culture produce α -subunit in the absence of GnRH, and α -subunit is found in the circulation in men with GnRH deficiency treated with thyroxine to suppress thyroid-stimulating hormone (TSH). Multiple elements in the promoter region contribute to α -subunit expression including tandem cAMP response elements in the human gene, a consensus GATA site and a steroidogenic factor-1 (SF-1) site (Barnhart & Mellon 1994, Maurer *et al.* 1999). The pituitary glycoprotein hormone basal element (PBGE) is important in basal transcription through interaction with LIM homeodomain proteins (Heckert *et al.* 1996), but how basal expression is maintained at a high level is not well understood. There is little effect of activin on α -subunit expression (Attardi *et al.* 1989). Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates α -subunit transcription by activating a G-protein receptor that couples primarily to cAMP/protein kinase A (PKA) (Tsuji *et al.* 1995, Fowkes *et al.* 2001). PACAP is expressed in the hypothalamus (Masuo *et al.* 1994) and the embryonic pituitary (Skoglosa *et al.* 1999), but in the adult pituitary is present in low levels (J Moore, unpublished observations), and its role as a paracrine regulator of gonadotrophs remains to be clarified.

The number of GnRH-receptors is an important determinant of gonadotropin secretion. Activin stimulates GnRH-receptor synthesis (Braden & Conn 1992) and increases the number of gonadotrophs that are GnRH responsive (Childs & Unabia 1997), and up-regulation is blocked by inhibin (Braden *et al.* 1990, Winters *et al.* 1996) or follistatin (Fernandez-Vazquez *et al.* 1996, Duval *et al.* 1999). There is an activin-response element within -387/-308 of mouse GnRH-R promoter (Norwitz *et al.* 2002a), and electrophoretic mobility shift assay experiments using α T3-1 nuclear extract or SMAD, Jun, and Fos proteins demonstrated direct binding of activator protein complex-1 (AP-1) (Fos/Jun) protein complexes to (327/322, and SMAD proteins to (329/328 (Norwitz *et al.* 2002b). While activin was found to promote GnRH-mediated transcriptional activation of the mouse GnRH-R gene (Norwitz *et al.* 2002a), no promoter activation was found with activin alone, suggesting that increased receptor synthesis in activin-treated pituitary cells in the absence of GnRH could represent a post-transcriptional effect. PACAP renders gonadotrophs more responsive

to GnRH (Tsujii *et al.* 1994). PACAP stimulates the GnRH promoter (Pincas *et al.* 2001) and may regulate the expression of the GnRH-R gene.

Paracrine control of gonadotropins and pubertal maturation in the male rat

The pituitary gonadotropins are differentially regulated during maturation in the male rat. Between postnatal days 20 and 30, serum FSH rises to levels that exceed those of adult rats while there is little or no change in LH concentrations (Swerdlow *et al.* 1971, Payne *et al.* 1977, Ketelslegers *et al.* 1978, Merry & Holehan 1981). Figure 1 demonstrates that this disparity is due to the differential expression of the gonadotropin subunit genes, and that GnRH-receptor mRNA increases in parallel with FSH- β (Moore *et al.* 2003, Zapatero-Caballero *et al.* 2003). While the mechanism controlling the selective rise of these mRNAs has not yet been thoroughly elucidated, up-regulation was blocked by a GnRH antagonist (Zapatero-Caballero *et al.* 2003), and both genes are stimulated by activin.

A role for inhibin in the control of FSH during postnatal development in the male rat was proposed from the rise in circulating FSH that followed immuno-neutralization with an α -inhibin antiserum in juvenile rats that was lost after puberty (Culler & Negro-Vilar 1988, Rivier *et al.* 1988). Immunoreactive inhibin was subsequently reported to decline coincident with the rise in FSH (Ackland & Schwartz 1991). Although the inhibin immunoassay used did not discriminate between the bioactive inhibin α - β dimer and the uncombined inhibin- α subunit that circulates in excess, results with specific two-site ELISAs for inhibin-B (Woodruff *et al.* 1996, Sharpe *et al.* 1999) tend to confirm those findings.

Other factor(s) may contribute to the differential regulation of the gonadotropins during the juvenile period. We studied pituitary follistatin mRNA expression (Moore *et al.* 2003) and found no significant change in the mRNA species encoding the larger and more abundant follistatin-315 peptide between ages 20 and 30 days. By contrast, the mRNA encoding the more potent follistatin-288 is significantly reduced from day 20 to 30. This reduction is

Figure 1 Developmental changes in mRNA expression in male rats. Data from Moore *et al.* 2003.

reciprocal to the increased level of expression of FSH- β mRNA, and is followed by an increase in follistatin-288 mRNA that is coincident with the decline in FSH- β mRNA expression to adult levels. Therefore, changes in follistatin expression may be responsible for increases and decreases in pituitary activin tone, and synergize with inhibin to influence gonadotropin subunit and GnRH-receptor gene expression during sexual maturation.

Paracrine regulation of gonadotrophs in male primates

The activin–inhibin–follistatin system also regulates gonadotrophs in primates. Activin stimulates FSH secretion and FSH- β mRNA in pituitary cell cultures from human abortuses and adult male rhesus monkeys (Blumenfeld & Ritter 2001, Kawakami *et al.* 2002), and up-regulation of GnRH-receptors by activin is inferred from the rise in GnRH-stimulated FSH and LH secretion that followed rh-activin-A infusion in cynomolgus monkeys (McLachlan *et al.* 1989). FSH secretion by human and non-human primate pituitary cultures is suppressed by inhibin (Fingscheidt *et al.* 1998, Blumenfeld & Ritter 2001). But unlike in rats, FSH- β gene expression and FSH secretion is not sustained in monkey pituitary cultures when GnRH is absent, in part because of follistatin production by proliferating folliculostellate cells (Kawakami *et al.* 2002).

FSH secretion *in vivo* is also more dependent on GnRH in male primates than in rats. Prepubertal boys, in whom GnRH is secreted at low levels, and men with a complete form of congenital hypogonadotropic hypogonadism have low plasma levels of both FSH and LH. Furthermore, the level of expression of the mRNAs for both FSH- β and LH- β is very low in prepubertal monkeys (S Winters and T Plant, unpublished observations). Circulating levels of FSH are low in prepubertal boys with bilateral cryptorchidism but are increased in adults (Christiansen *et al.* 2002). GnRH antagonists reduce circulating FSH as well as LH in normal adult men, although suppression of LH may be proportionately greater (Herbst *et al.* 2002). The anencephalic fetus, which lacks a hypothalamus, produces glycoprotein α -subunit but little or no FSH- β or LH- β subunits (Hagen & McNeilly 1975). Finally, the switch from a positive to a negative correlation between circulating levels of inhibin-B and FSH during human puberty (Andersson *et al.* 1998), and the suppression of FSH- β mRNA when orchidectomized adult monkeys are treated with inhibin (Majumdar *et al.* 1995) imply that the activin/inhibin system for the paracrine regulation of FSH in primates becomes operational as GnRH increases.

There is evidence that pituitary autocrine/paracrine factors may explain the markedly greater post-orchidectomy rise in FSH that occurs in male primates when compared with rats. Whereas plasma FSH levels increase 1- to 4-fold following orchidectomy in rats (Rodi *et al.* 1990), FSH levels in long-term-castrated rhesus monkeys (Attardi *et al.* 1992) and orchidectomized human males are

50- to 100-fold increased. These increases are paralleled by proportionate increments in FSH- β mRNA concentrations: in rats, orchidectomy increases FSH- β mRNA levels 2- to 4-fold whereas in the monkey, pituitary FSH- β mRNA is increased 20- to 40-fold by castration (Fig. 2). Species differences in pituitary follistatin expression contribute to these differing FSH responses. In rats, pituitary follistatin expression gradually increases over 2 weeks following orchidectomy to peak levels up to 25-fold higher than those of intact male rats (Kaiser & Chin 1993). FSH- β mRNA levels rise following orchidectomy in rats to peak increments of 2- to 4-fold at 7–10 days, which in many studies is followed by a decline to values that are similar to, or only slightly higher than intact controls by day 28. Although the castration-associated rises in FSH- β (Rodin *et al.* 1989) and follistatin mRNAs (Dalkin *et al.* 1998) are each GnRH dependent, the more gradual rise in follistatin expression has not been explained. Overall, the time course of the changes in follistatin and FSH- β mRNAs suggest that follistatin acts to restrain FSH- β gene expression subsequent to orchidectomy in male rats.

In primates, by contrast, the results in Fig. 3 reveal that follistatin mRNA levels are unaffected by bilateral orchidectomy (Winters *et al.* 2001), and it is therefore tempting to propose that the large 50-fold increase in FSH- β mRNA levels and FSH secretion occur partly because there is no rise in follistatin to restrain FSH- β gene expression. Insight into this hypothesis derives from experiments *in vitro* in which GnRH was shown to increase follistatin expression in pituitary cell cultures from rats but not from monkeys (Kawakami *et al.* 2002). This finding could be explained

Figure 2 A comparison of the effects of orchidectomy on gonadotropin subunit mRNA levels in adult male monkeys and rats. From Winters *et al.* 2001.

Figure 3 Pituitary follistatin mRNA concentrations in intact and orchidectomized adult male monkeys and rats. Data from Winters *et al.* 2001.

by species differences in the pituitary cells that express follistatin, or by differences in GnRH signaling pathways that activate follistatin expression in gonadotrophs.

GnRH pulse frequency is believed to differentially regulate LH and FSH production. Experiments in ovariectomized GnRH-deficient monkeys first demonstrated that GnRH at slow frequencies favors FSH over LH secretion (Wildt *et al.* 1981). Subsequently, rapid-frequency GnRH pulses administered to ovariectomized ewes in which GnRH was suppressed by progesterone treatment were found to increase α -subunit and LH- β more than FSH- β mRNA (Leung *et al.* 1987). Experiments in male rats (Dalkin *et al.* 1989, Kirk *et al.* 1994) and in pituitary cell cultures from orchidectomized testosterone-replaced male rats (Besecke *et al.* 1996) have shown that rapid GnRH pulse frequencies (e.g. every 15–30 min) favor LH- β over FSH- β gene expression. GnRH applied continuously (Attardi *et al.* 1989) or as rapid-frequency pulses (Besecke *et al.* 1996, Dalkin *et al.* 1999) up-regulates follistatin mRNA in this species. The consequence is a presumed blockade by follistatin of activin-stimulated FSH- β gene expression.

Since follistatin in the primate pituitary does not appear to be increased by GnRH, how GnRH pulse frequency might regulate FSH and LH production differentially in primates is less clear. In orchidectomized adult male monkeys rendered gonadotropin deficient by arcuate lesions, GnRH replacement at 1 pulse per hour produced higher LH but similar FSH levels to GnRH every 3 h, suggesting that GnRH pulse frequency is an important determinant of LH but not of FSH secretion in male primates (Plant & Dubey 1984). In men with congenital hypogonadotropic hypogonadism who were treated long-term with pulsatile GnRH, increasing the frequency of GnRH stimulation from every 2 h to every 30 min for 7 days increased serum LH levels 3-fold, and FSH levels rose by 50% (Spratt *et al.* 1987) and in a similar population of men, increasing the GnRH pulse frequency from every 1.5 h to every 0.5 h suppressed plasma FSH but plasma LH levels were unchanged (Gross *et al.* 1986). Gonadotrophs are known to secrete less FSH than LH in response to stimulation by GnRH, and in each

Figure 4 Follistatin and FSH- β mRNA levels in primary pituitary cell cultures from adult male rhesus monkeys treated with test substances for 1 or 3 days. From Kawakami *et al.* 2002. * $P < 0.05$ vs vehicle treated cultures, adjusted to 100%.

of the studies in men, the interpretation of the results is further complicated by changes in testosterone, estradiol and inhibin-B as a consequence of increased GnRH.

Species differences in pituitary follistatin may also explain why FSH regulation by androgens differs in primates and rats. When androgens are administered to rats,

FSH secretion decreases because GnRH and GnRH-R are suppressed, however LH is suppressed more effectively in castrated rats than is FSH (Swerdlow *et al.* 1973). While there are several potential explanations for this difference in feedback control, testosterone was subsequently found to increase FSH secretion directly (Mittler 1974) by stimulating FSH- β mRNA (Gharib *et al.* 1990), an effect which tends to offset feedback inhibition of GnRH and its receptor. The rise in FSH- β during androgen treatment in the absence of GnRH is partly due to suppression of follistatin (Bilezikjian *et al.* 1996) which allows activin to upregulate the FSH- β gene. Testosterone was also shown recently to increase FSH- β mRNA primary transcripts in rat pituitary cell cultures treated with follistatin (Burger *et al.* 2004) implying a direct effect on rat FSH transcription, as well as to activate the ovine FSH- β promoter in L β T-2 cells (Spady *et al.* 2004). On the other hand in monkeys (Khurshid *et al.* 1991) and men, the combination of testosterone and a GnRH antagonist reduced serum FSH levels more effectively than a GnRH antagonist alone, and testosterone enanthate suppressed circulating FSH levels in untreated GnRH-deficient men (Winters 1994). Moreover, unlike in rats, follistatin expression in primate pituitary cell cultures is stimulated by testosterone, and FSH- β mRNA is suppressed (Kawakami *et al.* 2002) (Fig. 4). Androgens decreased human FSH- β mRNA levels in pituitary cells from mice expressing this human transgene (Kumar & Low 1995) suggesting that species-specific factors in the pituitary cellular environment rather than motifs in the hFSH- β promoter are responsible for the differences in FSH regulation by testosterone in rodents and primates.

Gonadal steroids also affect GnRH-stimulated LH and FSH secretion and gonadotropin subunit gene expression differently in male primates and rats. Testosterone suppresses, and estradiol increases GnRH-stimulated LH and

Figure 5 Effects of testosterone or estradiol on GnRH-stimulated LH secretion by cultured pituitary cells from adult male monkeys (A and B) or rats (C and D). Cells were treated with 10 nM testosterone (A and C) or 0.1 nM estradiol (B and D) beginning 48 h before the perfusion. Pulses of 10 nM GnRH were administered hourly. From Kawakami & Winters 1999.

FSH secretion by pituitary cells from rats (Labrie *et al.* 1978) whereas in monkey pituitary cells, we (Kawakami & Winters 1999) found no significant effect of testosterone or dihydrotestosterone, while for estradiol, an initial period of enhancement was followed by significant suppression of GnRH-stimulated gonadotropin release (Fig. 5). These differences are partly through GnRH-receptors that in the rat are increased by estradiol (Emons *et al.* 1988) and downregulated by androgens (Giguere *et al.* 1981). While less is known about GnRH-receptors in the male primate, pituitary mRNA levels are decreased by estradiol while androgens appear to be without effect (Kawakami & Winters 1999). α -Subunit mRNA levels are also directly suppressed by androgens in pituitary cells from rats but not primates. Androgen receptors are expressed in primate gonadotrophs, and appear to traffic normally (Okada *et al.* 2003), and the resistance of primate gonadotrophs to androgens remains unexplained.

Finally, inhibin-B appears to play a greater role in the regulation of FSH- β gene expression in adult monkeys than in rats. Specifically, while immunoneutralization of circulating inhibin failed to increase FSH secretion in adult rats (Culler & Negro-Vilar 1988, Rivier *et al.* 1988), this experimental approach increased FSH in adult monkeys (Medhamurthy *et al.* 1991). Interestingly, however, circulating inhibin levels in rats and monkeys tend to overlap (Sharpe *et al.* 1999). Perhaps because the current two-site inhibin-B assays utilize antisera against the human inhibin subunits, and there are large-molecular-weight forms of circulating inhibin-B of uncertain bioactivity, at least in primates (Robertson *et al.* 1996, Winters & Plant 1999), immunoassay detection and specificity may differ in primates and rats. If so, the greater rise in FSH following castration or immunoneutralization of inhibin in male primates could reflect higher circulating levels of bioactive inhibin-B.

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