

Molecular Aspects of Steroid Hormone Action in the Male Reproductive Axis

Minireview

MARGARET A. SHUPNIK AND
DEREK A. SCHREIHOFFER

*From the Division of Endocrinology and Metabolism,
Department of Internal Medicine, University of Virginia
Health Sciences Center, Charlottesville, Virginia.*

Maintenance of normal reproductive function is dependent on the coordinated release of hormones in the hypothalamic-pituitary-gonadal cascade. Gonadotropin-releasing hormone (GnRH) is released intermittently into the pituitary portal blood from neuroendocrine cells in the basal hypothalamus and acts to stimulate gonadotropes in the anterior pituitary to synthesize and release two peptide hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), into the general circulation. Follicle-stimulating hormone and LH are glycoproteins that consist of two subunits, an alpha subunit, which is also shared by pituitary thyroid-stimulating hormone and placental chorionic gonadotropin in humans, and unique beta subunits that confer biological activity. All the subunits are encoded by separate genes on different chromosomes, permitting differential control of gene activity. The genes for rodent and human gonadotropin subunits have been cloned and characterized and used to investigate mechanisms of hormonal regulation. The gonadotropins act to maintain gonadal function, including germ cell formation in males, ovarian cyclicity in females, and steroid (androgens, estrogens, progestins) and gonadal peptide (activins, inhibins) production in both sexes. The cascade is maintained by steroid and peptide feedback at both the hypothalamic and pituitary level (Fig. 1).

Steroid Hormone Receptors

Actions of the reproductive hormones are dependent on the proper expression of receptors in target tissues. The two classes of hormones involved in reproductive function act through different mechanisms. The peptide hormones GnRH, LH, FSH, activin, and inhibin act via cell-surface receptors. Hormone binding to receptors activates ion channels and second-messenger signaling pathways that alter both hormone secretion and gene ex-

pression from target cells. In contrast, steroid hormone receptors belong to a family that includes thyroid hormone, vitamin D, and retinoic acid receptors and that is localized to the cell nucleus and acts directly as ligand-activated transcription factors (Evans, 1988). The lipophilic nature of steroid hormones allows them to pass into the nucleus where they are able to bind to their specific receptor. The hormone-receptor complexes then dimerize and bind specific DNA recognition sequences (hormone-response elements) in the promoter regions of steroid-regulated genes. Once bound, these complexes promote structural changes in DNA and interact with proteins involved in transcription to enhance or inhibit gene expression. Estrogen (E) and testosterone (T) bind to specific estrogen and androgen receptors (ER and AR, respectively) that recognize different DNA sequences (Fig. 2). Thus, E and T would not be assumed to act identically or to have the same target genes. Recent studies have identified different isoforms and splice variants for both AR and ER, thus providing another level of regulation for steroid-responsive genes.

Steroid Hormones and Sexual Development

Fetal testes, but not ovaries, express the androgen synthetic enzyme 3- β -hydroxysteroid dehydrogenase, thus conferring to testes the exclusive ability to produce T. Testosterone, its 5 α reduced form, dihydrotestosterone (DHT), and Müllerian-inhibiting substance (MIS) from the fetal testes are important determinants of sexual phenotype. Müllerian-inhibiting substance prevents the formation of the female Müllerian duct system and thus prevents the female urogenital phenotype. However, expression of a male phenotype is dependent on secretion of T and expression of AR. For example, removal of the gonads in indifferent rabbit embryos leads to a female phenotype (Jost, 1972). Similarly, treatment of rat embryos with antiandrogens prevents expression of a male phenotype (Neumann et al, 1970). Defects in T synthesis from fetal testes can also lead to varying degrees of incomplete virilization in humans, up to and including expression of a female phenotype in genetic males (Wilson and Goldstein, 1975). However, since production of MIS is normal, no uterus or fallopian tubes are formed. Androgen receptor mutations lead to a similar testicular feminization (Tfm) phenotype. With this X-linked mutation, genetically male mice have normal testes in the abdomen and produce MIS and T but have no virilization in the

Correspondence to: Dr. Margaret A. Shupnik, Box 578, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908.

Received for publication January 31, 1997; accepted for publication April 4, 1997.

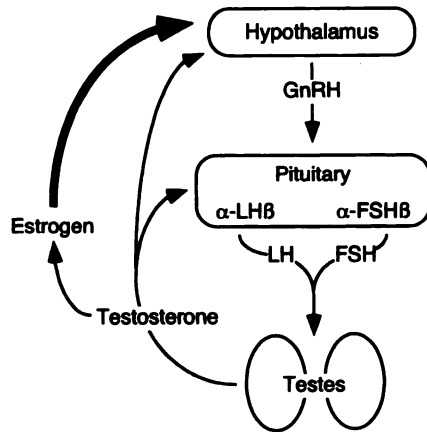


FIG. 1. Feedback sites of estrogen (E) and testosterone (T) on the hypothalamic-pituitary axis.

urogenital tract (Quigley et al, 1995). Similar androgen insensitivity has been well characterized in men (Quigley et al, 1995). No direct effect of E in male sexual development has been defined, although in adults both T and E have important roles in the regulation of gonadotropin levels and normal fertility.

Steroid Feedback on the Gonadotropin Axis—Hypothalamic vs. Pituitary Action

Steroid modulation of the gonadotropin axis has been studied most intensively in rodent models in which gonadectomy dramatically elevates levels of serum LH and FSH, and E or T replacement suppresses these levels to those of intact animals (Gharib et al, 1990b). These changes may result from removal of negative feedback at the level of the pituitary or, indirectly, at the level of the hypothalamus. Changes in sex steroid levels result in variations in GnRH pulse frequency and amplitude, which alter gonadotropin secretion and subunit mRNA levels (Gharib et al, 1990b; Haisenleder et al, 1990; Shupnik, 1990). At the level of the pituitary, hormonal modulation may occur by changes in GnRH or steroid receptor number or subtype, thus altering or enhancing pituitary responsiveness to hormones, or by direct modification of gonadotropin gene activity.

The rise in serum gonadotropins 1–2 weeks after steroid removal is mirrored by increases in both steady-state mRNA levels and transcription rates of the gonadotropin genes (Gharib et al, 1990b) and occurs more rapidly in males (3–7 days) than in females (21 days) (Gharib et al, 1987; Wierman and Wang, 1990). Administration of E or T to males first decreases elevated gonadotropin gene transcription within 1 hour, but complete suppression requires between 1–3 days of treatment. The long time-course suggests indirect effects on the hypothalamic-pituitary axis, and this view is supported by the failure of E or T to suppress gonadotropin secretion, mRNA levels,

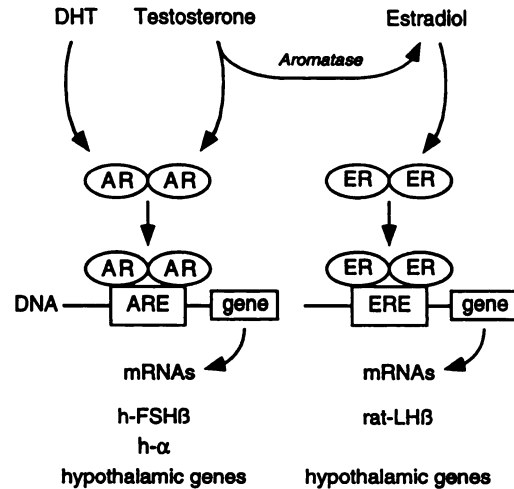


FIG. 2. Modulation of specific genes bearing either androgen-response elements (ARE) or estrogen-response elements (ERE) in their promoter regions. Regulation occurs by binding of ligand to specific androgen and estrogen receptors (AR and ER) directly to the DNA sequence. Estradiol and dihydrotestosterone (DHT) exert their actions only through ER or AR, respectively, but testosterone may be converted to estradiol and act through both receptor pathways.

or gene transcription in isolated pituitary cells. In fact, E treatment specifically stimulates rat LH β gene transcription directly via an ER-binding site in the gene promoter, while having no effect on the α -subunit or FSH β genes (Shupnik and Rosenzweig, 1991). Parallel studies with mouse, rat, and human gonadotropin promoters in reporter-gene constructs have found no direct suppressive effects of E on promoter activity in transfected pituitary cells or cell lines (Keri et al., 1991).

Definitive demonstrations of negative steroid feedback at the hypothalamic level has come from transgenic mouse experiments using gonadotropin reporter-gene constructs and the promoters for rat LH β (Fallest et al, 1995), bovine LH β (Keri et al, 1994), and human α (Keri et al, 1991) genes. The resultant transgenic animals expressed the reporter enzymes in pituitary cells; promoter activity was increased after castration and restored to levels in intact controls after injections of E to males and females and T or DHT to males. Treatment with GnRH antagonists prevented the castration-induced rise in promoter activity for all three genes, and inhibitory steroid effects could be overcome by pulsatile administration of GnRH. Because the gonadotropin promoters used cannot respond to steroids directly, suppression of reporter-gene activity must have occurred via extrapituitary mechanisms and probably by alterations in GnRH pulses. This interpretation is supported by *in vivo* studies in male rats in which suppression of GnRH pulses, gonadotropin secretion, and subunit mRNA levels by T implants could be overcome by exogenous GnRH pulses (Haisenleder et al, 1990). Thus, negative steroid feedback in rats appears to occur primarily at the level of the hypothalamus. Be-

cause the GnRH neurons themselves appear to have no or at least very few ER or AR, the mechanisms by which pulses are altered is presently unknown.

Steroids act directly on pituitary cells to modulate the numbers, or the subtypes, of steroid and GnRH receptors. Several groups have demonstrated that E treatment increases pituitary GnRH-receptor number (Kaiser et al, 1993; Colin et al, 1996) and that increased receptor density results in increased differential responsiveness of the gonadotropin subunit genes *in vitro* (Kaiser et al, 1995). Orchidectomy had no effect on the responsiveness of cultured male rat pituitary cells to GnRH, but positive T effects could occur via aromatization to E. Modulation of steroid responsiveness also occurs with changes in steroid treatment. Estrogen and T, but not DHT, administration induces a truncated, pituitary-specific form of ER, which may modulate E-induced gene transcription (Friend et al, 1995). Thus, steroids may be increasing pituitary sensitivity to both GnRH and E while altering GnRH pulses and the overall physiological response results from integration of these pathways.

Estrogen and Testosterone—Identical and Divergent Feedback Pathways

Estrogen and T effects in whole animals are often identical, particularly with T-E conversion in target tissues. However, some divergent biological actions have been noted with T and DHT, particularly on FSH secretion and the FSH β gene and may be important under specific physiological circumstances. Serum FSH is higher in intact male than in female rats, and the castration effect on serum FSH and FSH β mRNA is much less than on LH and LH β . In contrast to the suppressive effects of E on all three subunit mRNAs in castrated males, T and DHT selectively fail to suppress FSH β mRNA *in vivo*. Because androgens act directly at the level of the hypothalamus to decrease GnRH pulses, these data could be explained by opposing effects of androgens on the hypothalamus versus the pituitary. Treatment of cultured male rat pituitary cells with T or DHT selectively and specifically stimulate FSH secretion and FSH β mRNA (Gharib et al, 1990a); similar results were obtained *in vivo* in male rats treated with a GnRH antagonist (Wierman and Wang, 1990). This process appears to occur by some stimulation of gene transcription and significant decreases in mRNA turnover, resulting in a much longer FSH β mRNA half-life. Thus, elimination of hypothalamic influences revealed positive subunit-specific feedback of T at the pituitary level. Fertility in males might thus be maintained with high physiological levels of T. However, an important species-specific difference in steroid regulation exists between the rodent and human FSH β subunit genes. In male transgenic mice bearing regulatory and coding regions of the hFSH β (human) gene, castration increases expression of

both endogenous mFSH β (mouse) and transgenic hFSH β genes, but treatment of these animals with T or E suppresses hFSH β selectively. Treatment of cultured pituitary cells from these male transgenic mice with steroids and *in vivo* experiments in hypogonadal mice containing the human transgene demonstrates that the human gene is directly suppressed by T and DHT, even without changes in GnRH (Kumar and Low, 1995). These data, coupled with the demonstration of an androgen-sensitive suppressive element in the human α -subunit gene (Clay et al, 1993), suggest that in humans androgens can act directly at the level of both the hypothalamus and the pituitary to suppress FSH synthesis.

Additional insight into the relative roles of E and T can be obtained from a number of genetic models or clinical cases in which the ER or AR are mutated. Androgen-resistant patients have elevated serum T and gonadotropin levels. Similarly, Tfm male mice have elevated gonadotropins and are completely infertile. Recent work has highlighted a surprising requirement for E in normal male physiology and fertility. Targeted disruption of the ER gene in mice results in complete infertility in homozygous males and females (Lubahn et al, 1993). Serum levels of E and LH are elevated, and the mRNAs for all three gonadotropin subunit genes are increased. In males, serum FSH is elevated, T levels are within the normal range, and testicular atrophy and seminiferous tubule dysmorphogenesis occur. Overall, these studies suggested little role for E in fetal development, some role for E in sexual development, and a major role for E in regulation of gonadotropin levels. Some data interpretation has been complicated by the recent discovery of a fully functional ER isoform, termed ER β , in rodents and humans (Kuiper et al, 1996). Estrogen receptor β is found at particularly high levels in testes, prostate, and ovaries, with significant levels of its mRNA in pituitary and other reproductive tissues. Specific developmental effects of E may be receptor specific, and tissue-specific effects of E may have at least some foundation in the presence of specific receptor subtypes. In humans, a single case of E resistance has been reported, in which a homozygous mutation of the ER gene results in a truncated, inactive protein (Smith et al, 1995). The 28-year-old male exhibited normal masculinization, with elevated E, LH, and FSH, and normal T serum levels. Fertility was not assessed; however, the patient had severe osteoporosis and tall stature due to failure of epiphyseal fusion. These results agree with those reported in patients with aromatase mutations that effectively abolish E synthesis. In one sibling pair with this defect, brother and sister had elevated T, LH, and FSH with tall stature, wrist osteopenia, and osteoporosis (Morimisha et al, 1995).

Summary

Both T and E play important roles in the regulation of reproductive function at the cellular and tissue levels in males. In addition to a causal role in the development of the male reproductive phenotype, androgens provide feedback regulation to the hypothalamus and to the pituitary in humans. Estrogen action is critical for normal bone fusion and mineralization in both men and women, and E action appears to play an important role in hypothalamic suppression of gonadotropin levels.

References

- Clay CM, Keri RA, Finicle AB, Hecker LL, Hamernik DL, Marsch KM, Wilson EM, French FS, Nilson JH. Transcriptional repression of the glycoprotein hormone α -subunit gene by androgen may involve direct binding of the androgen receptor to the proximal promoter. *J Biol Chem* 1993;268:13556–14564.
- Colin IM, Baurer-Dantoin AC, Sundaresan S, Kopp P, Jameson JL. Sexually dimorphic transcriptional responses to gonadotropin-releasing hormone require chronic *in vivo* exposure to estradiol. *Endocrinology* 1996;137:2300–2307.
- Evans R. The steroid thyroid hormone receptor superfamily. *Science* 1988;240:889–895.
- Falset PC, Trader GL, Darrow JM, Shupnik MA. Regulation of rat luteinizing hormone β gene expression in transgenic mice by steroids and a gonadotropin-releasing hormone antagonist. *Biol Reprod* 1995;53:103–109.
- Friend KE, Ang LW, Shupnik MA. Estrogen regulates the expression of several different estrogen receptor mRNA isoforms in rat pituitary. *Proc Natl Acad Sci USA* 1995;92:4367–4371.
- Gharib SD, Leung PCK, Carroll RS, Chin WW. Androgens positively regulate follicle-stimulating hormone β -subunit mRNA levels in rat pituitary cells. *Mol Endocrinol* 1990a;4:1620–1626.
- Gharib SD, Wierman ME, Badger TM, Chin WW. Sex steroid hormone regulation of follicle-stimulating hormone subunit messenger ribonucleic acid (mRNA) levels in the rat. *J Clin Invest* 1987;80:394–401.
- Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of the pituitary gonadotropins. *Endocr Rev* 1990b;11:177–199.
- Haisenleder DJ, Dalkin AC, Ortolano GH, Marshall JC, Shupnik MA. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes; evidence for differential regulation of transcription by pulse frequency *in vivo*. *Endocrinology* 1990;128:509–517.
- Jost A. A new look at mechanisms controlling sex differentiation in mammals. *Johns Hopkins Med J* 1972;130:38–53.
- Kaiser UB, Jaku Bowail A, Steinberger A, Chin WW. Regulation of rat pituitary gonadotropin-releasing hormone receptor mRNA levels *in vivo* and *in vitro*. *Endocrinology* 1993;133:931–934.
- Kaiser UB, Sabbaugh E, Katzenellenbogen RA, Conn PM, Chin WW. A mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone. *Proc Natl Acad Sci USA* 1995;92:12280–12284.
- Keri RA, Andersen B, Kennedy GC, Hamernik DL, Clay CM, Brace HD, Nett TM, Notides AC, Nilson JH. Estradiol inhibits the transcription of the human glycoprotein hormone α -subunit gene despite the absence of a high affinity binding site for the estrogen receptor. *Mol Endocrinol* 1991;5:725–733.
- Keri RA, Wolfe MA, Saunders TL, Anderson J, Kendall SK, Wagner T, Yeung J, Gorski J, Nett TM, Camper SA, Nilson JH. The proximal promoter of the bovine luteinizing hormone β -subunit gene confers gonadotrope-specific expression and regulation by gonadotrope-releasing hormone, testosterone, and 17 β -estradiol in transgenic mice. *Mol Endocrinol* 1994;8:1807–1816.
- Kuiper GGJM, Enmark E, Peltö-Huikko M, Nilsson S, Gustafsson J-A. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996;93:5925–5930.
- Kumar TR, Low MJ. Hormonal regulation of human follicle-stimulating hormone-beta gene expression: GnRH stimulation and GnRH-independent androgen inhibition. *Neuroendocrinology* 1995;61:628–637.
- Lubahn DB, Moyer JS, Golding JF, Couse K, Korach S, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 1993;90:11162–11166.
- Morimisha A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab* 1995;80:3689–3698.
- Neumann F, Berswordt-Wallarabe R, Elger W, Steinbeck H, Hahn JD, Kramer M. Aspects of androgen-dependent events as studied by antiandrogens. *Rec Prog Horm Res* 1970;26:337–410.
- Quigley CA, DeBellis A, Marschke KB, el-Awad MK, Wilson EM, French FS. Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocr Rev* 1995;16:271–321.
- Shupnik MA. Effects of gonadotropin-releasing hormone on rat gonadotropin gene transcription *in vitro*: requirement for pulsatile administration for luteinizing hormone- β gene stimulation. *Mol Endocrinol* 1990;4:1444–1450.
- Shupnik MA, Rosenzweig BA. Identification of an estrogen-responsive element in the rat LH β gene. *J Biol Chem* 1991;266:17084–17091.
- Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 1995;331:1051–1061.
- Wierman ME, Wang C. Androgen selectively stimulates follicle-stimulating hormone-beta mRNA levels after gonadotropin-releasing hormone antagonist administration. *Biol Reprod* 1990;43:563–571.
- Wilson JD, Goldstein JL. Classification of hereditary disorders of sexual development. *Birth Defects* 1975;11:1–16.