The relationships between gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and testosterone in males.

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INTRODUCTION

Observations that neutralization of gonadotropin-releasing hormone (GnRH) by administration of GnRH antiserum blocks the pulsatile luteinizing hormone (LH) secretion (Lincoln and Fraser, 1979; Ellis et al., 1983) and pulses of LH are synchronized to pulsatile GnRH release into hypophyseal portal blood (Caraty and Locatelli, 1988, Jackson et al., 1991) indicate that LH secretion in males is controlled by the hypothalamus through its secretion of GnRH.

GnRH, a decapeptide, released in a pulsatile manner from the median eminence into the hypophyseal portal circulation, binds to specific, high-affinity receptors on the membrane of pituitary gonadotropes (Hazum and Conn, 1988). The action of GnRH on LH release appears to be associated with several interacting post-receptor signal transduction pathways, including the Ca^{++}-pathway, the phospholipase pathway, the phopholipase A_2 and/or diglyceride lipase pathways, and the adenylate cyclase pathway.

LH, a glycoprotein, via the peripheral circulation, interacts with specific, high-affinity receptors on the plasma membrane of the interstitial Leydig cells within the testis (Dufau and Catt, 1978). The binding of LH to its receptors stimulates the membrane-bound adenylate cyclase (Mendelson et al., 1975). The increased intracellular cAMP levels then activate protein kinase in the cytoplasm of the Leydig cell (Dufau et al., 1977; Podesta et al., 1978) and through unidentified intermediate steps, eventually result in stimulation of the conversion of cholesterol to pregnenolone, which in turn enhances the synthesis of testosterone (Miller, 1988). Testosterone is either aromatized to estradiol or reduced to 5α-dihydrotestosterone.

It is well established that testosterone, a major androgenic steroid, exerts negative feedback to regulate LH secretion. In such a manner, removal of testosterone negative feedback by castration causes increased circulating mean LH concentration with more frequent pulses. However, because castration removes hormones other than testosterone, the hypothesis that testosterone alone or in combination with the metabolites of testosterone inhibits LH secretion should be elucidated.

Thus, the aim of this review is to explore factors that control the hypothalamo-pituitary-gonadal axis and their effects on LH in males.

Control of Luteinizing Hormone in Males)

1) Pattern of GnRH and LH secretions in males

Several studies using either portal cannulation or push-pull cannula perfusion techniques have demonstrated that, in males, GnRH is released in a pulsatile pattern (Dluzen and Ramirez, 1985; Caraty and Locatelli, 1988; Levine and Duffy, 1988; Jackson et al., 1991; Lin and Ramirez, 1991; Tilbrook et al., 1991). The minute to minute secretory pattern of GnRH pulses in the ewe was examined by Moenter et at. (1992). They found a square-wave contour of GnRH pulses that consisted of an abrupt increase (within 1 min of the onset of pulse), an elevated plateau of sustained (an average of 5.5 min) but fluctuating values, and a precipitous drop to pre-pulse levels within 3 min.

LH is secreted in a pulsatile pattern in males of many species including primates (Steiner et al., 1980; Plant, 1981), rats (Elllis and Desjardins, 1982; Levine and Duffy, 1988), and sheep (Sanford et al., 1974; Lincoln, 1976; D'Occhio et al., 1982).

Data from simultaneous measurement of GnRH and LH release in male (Caraty and Locatelli, 1988; Levine and Duffy, 1988; Jackson et al., 1991; Tilbrook et al., 1991) demonstrate that every LH pulse is preceded within 10 to 20 min by a pulse of GnRH, but not all GnRH pulses are associated with the corresponding LH pulses. In most cases, these "silent" GnRH pulses (GnRH pulses which do not cause LH pulses) are among the lowest in amplitude. Although the physiological significance of "silent" GnRH pulses is unknown, recent study suggests that it may potentiate the pituitary response to GnRH (Clarke and Cummins, 1987).

2) Pattern of testosterone secretion in males

Testosterone is secreted in an episodic pattern in males including primates (Steiner et al., 1980; Plant, 1981), rats (Elllis and Desjardins, 1982), and sheep (Sanford et al., 1974; Lincoln, 1976; D'Occhio et al., 1982). In all of these species, except rats, each testosterone pulse is preceded, within 20 to 60 min, by a discrete pulse of LH. Data from frequent measurement of testosterone release in intact rams demonstrate that testosterone increases gradually and reaches peak within approximately 1 h after pulse onset and then drops to pre-pulse levels within 3 to 4 h (D'Occhio et al., 1982).

3) Factors that control the hypothalamo-pituitary-gonadal axis and their effects on LH in males

A) Testosterone

I) The site of action of testosterone on LH secretion

The role and effect of testosterone may differ among species. In the male rat, there is controversy regarding to its role on GnRH secretion. Castration has shown increase (Eskay et al., 1977; Levine and Duffy, 1988), no change (Brar et al., 1985; Kalra et al., 1988), or decreased (Dluzen and Ramirez, 1985; Ching et al., 1987) GnRH secretion as compared in intact males. In the ram, there are several lines of indirect evidence that the hypothalamus may be the principal site for the inhibitory action of testosterone on LH secretion. Neutralization of GnRH by administration of GnRH antiserum blocks the pulsatile LH secretion (Lincoln and Fraser, 1979). The post-castration rise in LH is prevented in wethers implanted with testosterone, but a castration-type secretory pattern of LH is obtained in testosterone-implanted wethers with hourly GnRH infusion (Schanbacher and D'Occhio, 1984). Recently, two separate observations that castration increases (Caraty and Locatelli, 1988) and testosterone replacement decreases (Jackson et al., 1991; Tilbrook et al., 1991) GnRH pulse frequency as well as LH pulse frequency provide clear evidence that testosterone reduces LH pulse frequency by reducing GnRH pulse frequency. Thus, in the ram, the action of testosterone negative feedback primarily occurs at the hypothalamus. On the other hand, action of testosterone negative feedback at the pituitary in the ram is

debated. Testosterone or testosterone propionate fail to affect LH pulse amplitude (Jackson et al., 1991; Tilbrook et al., 1991). Furthermore, testosterone propionate does not significantly affect either mean LH concentrations or amplitude of LH pulses in hypothalamo-pituitary disconnected wethers receiving GnRH replacement (Tilbrook et al., 1991). In the male monkey, castration increases LH pulse frequency and testosterone replacement suppresses LH pulse frequency in short-term castrated male monkey (Plant, 1982). Furthermore, castration does not alter mean LH concentrations and amplitude of LH pulses in male monkeys which were radiofrequency-lesioned in the region of the arcuate nucleus (to abolish the endogenous GnRH secretion) and received frequent exogenous GnRH injections (Plant and Dubey, 1984). These findings suggest that the negative feedback action of testicular hormones plays no major role at the pituitary in this species.

Although the hypothalamus appears to be the primary site for the action of testosterone on LH secretion, testosterone decreases LH release directly at the pituitary level by reducing the pituitary responsiveness to GnRH *in vivo* (Debeljuk et al., 1974; Bremner et al., 1980; Kalra and Kalra, 1982; D'Occhio et al., 1983) and *in vitro* (Kamel and Krey, 1982; Kotsuji et al., 1988; Krummen and Baldwin, 1988). Testosterone suppresses LH release in vitro by modulating the post-GnRH receptor (Protein kinase C mediated) intracellular mechanisms (Hubert et al., 1988), Ca++ mobilization (Kamel and Krey, 1983), or arachidonic acid release (Kamel and Kubajak, 1988). Also testosterone depresses GnRH stimulation of G protein GTPase (Ravindra and Aronstam, 1992) and decreases pituitary GnRH receptor concentrations (Clayton and Catt, 1981; Frager et al., 1981; Limonta et al., 1986). However, hypothalamic lesion (Clayton et al., 1982a; Pieper et al., 1982) or immunoneutralization of endogenous GnRH with GnRH antiserum (Clayton et al., 1982b) prevent the castration-induced increases in GnRH receptor concentrations, and GnRH replacement reverses the effect of hypothalamic lesion on GnRH receptor (Clayton et al., 1982a). Thus, the inhibitory effect of testosterone on GnRH receptor may be indirect by reducing GnRH secretion at the hypothalamus.

II) The site of action of testosterone on LH synthesis

Studies on changes of hypothalamic GnRH messenger ribonucleic acid (mRNA) in male rats following castration also have shown contradictory results. Castration reportedly results

in an increase (Toranzo et al., 1989; Selmanoff et al., 1991), no change (Wiemann et al., 1990) or a decrease (Rothfeld et al., 1987; Park et al., 1988) in cellular levels of GnRH mRNA. In male hamsters, castration fails to alter hypothalamic GnRH mRNA levels (Ronchi et al., 1992). Testosterone may be involved in the processing of GnRH from its precursor, pro-GnRH. Castration increases the pro-GnRH content but decreases the GnRH content, and testosterone reverses those effects in male rats (Roselli et al., 1990), suggesting that testosterone may promote the enzymatic processing of pro-GnRH to GnRH.

Testosterone suppresses LH-subunit mRNA levels and LH-subunit apoprotein synthesis at the pituitary. Castration results in increased mRNA levels of both LH-α and LH-β subunits (Abbot et al., 1985; Gharib et al., 1986; Kitahara et al., 1990) as well as LH-subunit apoprotein synthesis (Vogel et al., 1986). Using in situ hybridizaztion and immunocytochemical techniques, Childs et al. (1987) reported that castration increased the amount of LH-β mRNA per pituitary cell as well as the number of LH-β mRNA containing cells in male rats. Testosterone replacement suppresses both LH-subunit mRNA levels (Gharib et al., 1986; Abbot et al., 1988) and LH-subunit apoprotein synthesis (Krummen and Baldwin, 1988). However, administrations of GnRH antagonist or GnRH antiserum in castrated rats suppress the castration-induced increases in both LH-subunit mRNA levels to intact levels (Lalloz et al., 1988; Kato et al., 1989), whereas pulsatile injections of GnRH increase steady state levels of both LH-subunit mRNA (Haisenleder et al., 1988; Dalkin et al., 1989). In addition, GnRH increases LH-subunit apoprotein synthesis (Starzec et al., 1986; Krummen and Baldwin, 1988). Thus, testosterone may suppress LH-subunit mRNA levels and LH-subunit apoprotein synthesis either directly at the pituitary or indirectly by reducing GnRH secretion at the hypothalamus.

III) The mode of action of testosterone on LH secretion

In females, autoradiographic studies fail to demonstrate estradiol binding sites in GnRH cells (Shivers et al., 1983; Watson et al., 1992). Instead, estradiol binding sites are observed in a number of neurotransmitter and neuropeptide producing cells, including catecholaminergic, GABAergic and neuropeptide-Y producing neurons, which have been reported to have connection with GnRH neurons (Sar, 1983; Flugg et al., 1986; Sar et al., 1990). Thus, these observations raise the hypothesis that the effect of estradiol on GnRH

secretion in females may be mediated via these steroid receptor containing neurons. Although androgen-binding neurons are widely distributed in the male brain including the regions which are rich in GnRH cell bodies (Sar and Stumpf, 1975), no direct evidence for the presence of androgen binding sites in GnRH cells has been reported in the male of any species. Therefore, description of the neuronal systems which may mediate the effect of testosterone on hypothalamo-pituitary axis in males relies on strictly observations from studies on females.

The effect of dopamine (DA) on LH secretion is controversial. In male rats, DA stimulates GnRH release in vitro (Negro-Vilar et al., 1979) as well as LH release (Kamberi et al., 1970) or suppresses LH release (Gallo, 1980). In castrated rams, intraventricular infusion of DA does not affect LH secretion (Riggs and Malvin, 1974b). Either intravenous or intracerebroventricular injection of the dopaminergic D_2 antagonist pimozide fail to prevent the suppressive effect of testosterone on LH secretion in castrated rams (Tilbrook and Clarke, 1992). Thus, these findings suggest that DA may not be involved in the action of testosterone negative feedback on LH secretion in the ram.

Inhibitory effects of testosterone on LH secretion in male rats remain unaltered after removal of hypothalamic norepinephrine by either transection of the ascending noradrenergic system or intracerebroventricular infusion of 6-hydroxydopamine (Maccalla et al., 1987). Thus, it is not likely, at least in male rats, that the noradrenergic system is involved in regulation of LH in response to testosterone negative feedback.

Intraventricular infusion of serotonin suppresses LH secretion (Riggs and Malvin, 1974b). However, whether testosterone negative feedback mechanism is mediated via this system remains to be known.

The opiodergic system may be involved in regulation of LH secretion by testosterone. Morphine decreases and the opioid antagonist naloxone increases LH secretion in testosterone-treated castrated rams (Lincoln et al., 1987). Similar results have been reported in male hamsters (Roberts et al., 1985).

IV) The effect of patterns of testosterone on LH secretion

Although the effects of testosterone on LH secretion have been extensively studied, in most previous studies, constant levels of testosterone were administered by either

implantation of Silastic tubes filled with testosterone, continuous of testosterone, or periodic injection of testosterone propionate. All of these procedures produce relatively constant circulating testosterone concentrations. Since testosterone is secreted in an episodic pattern, constant testosterone administration does not reflect the endogenous testosterone secretory patterns. The significance of pulsatile pattern of testosterone in regulation of LH has been proposed in studies on the monkey, in which constant testosterone replacement fails to suppress LH secretion (Resko et al., 1977), whereas pulsatile testosterone replacement suppresses LH secretion (Plant et al., 1978). Because dose of testosterone used in latter study was 2 to 3 times higher than that in former study, it is difficult to compare the effects of pulsatile pattern of testosterone with those of constant pattern on LH secretion. On the other hand, the postulate that a constant testosterone may be more effective than a pulsatile testosterone in suppressing LH secretion has been raised from the observations that constant replacement of castrated animals with only 1/2 to 1/3 of intact levels of testosterone causes suppression of LH concentrations to the intact levels (Damassa et al., 1976; Moger, 1976; D'Occhio et al., 1983). More effective suppression of LH by a constant pattern of testosterone than a pulsatile pattern of testosterone has been suggested by Hutchison and Goldman (1975). Although systemic comparison of the effects of these two patterns of testosterone on LH secretion has not been studied, they found that LH concentrations were much lower in short-term castrated rats receiving continuous testosterone infusion for 24 h in one experiment than those in animals receiving single bolus injection of same amount of testosterone in another experiment.

Conclusive evidence that a constant pattern is more effective has been provided by the study (Rhim et al., 1993), in which constant testosterone infusion suppressed LH secretion more than a quasi physiological pulsatile pattern of same amount of testosterone in castrated rams.

B) Estradiol

Estradiol is formed from testosterone by aromatization in the testis (Longcope et al., 1972), brain (Naftolin et al., 1975), and a variety of peripheral tissues (Longcope et al., 1978). Treatment with aromatase inhibitor stimulates LH secretion in men (D'Agata et al., 1981), monkeys (Ellinwood et al., 1984), and rams (Schanbacher, 1984), and this effect is blocked

by concurrent treatment with estradiol (Ellinwood et al., 1984), indication that estradiol formation plays an important role in the regulation of LH secretion.

The action of estradiol on the hypothalamo-pituitary axis has been implied by the demonstration of estradiol receptors in the hypothalamus and pituitary in males including rats (Kato, 1975; Lieberburg and McEwen, 1977) and rams (Thieulant and Pelletier, 1979; Pelletier and Caraty, 1981). Estradiol suppresses circulating LH concentrations in intact males (Bolt, 1971; de Jong et al., 1975) and castrated animals (Riggs and Malven, 1974b; Steiner et al., 1976; Schanbacher and Ford, 1977; Sanford and Robaire, 1990). Conversely, active immunization against estradiol causes increased LH secretion in intact male rats (Nishihara and Takahashi, 1983) and intact rams (Schanbacher, 1984), while passive immunization of intact rams, however, is associated with only moderately increased LH secretion (Sanford, 1987). Combined with the observations that estradiol prevents the increases in pituitary GnRH receptors after castration in male rats (Clayton and Catt, 1981; Conne et al., 1982), those data suggest that estradiol may play an important role in the regulation of LH secretion at the level of hypothalamus and/or pituitary.

In addition, Kalra and Kalra (1980) reported that estradiol increased medial basal hypothalamic GnRH content, whereas Toranzo et al. (1989) reported that estradiol prevented the post-castration rise in pro-GnRH mRNA levels in castrated male rats. These observations, although they contradict each other as do the studies in changes of hypothalamic GnRH mRNA following castration in male rats, provide further evidence that estradiol may affect GnRH synthesis as well. However, a study using an aromatase inhibitor has demonstrated that the inhibitory effect of testosterone on LH secretion (Marynick et al., 1979) may be independent of its aromatization.

C) 5α-dihydrotestosterone

Testosterone is converted by 5α-reductase into the more active androgen, 5α-dihydrotestosterone (DHT) which interacts more efficiently with the androgen receptor (Anderson and Liao, 1968). Several studies have demonstrated that 5α-reductase activity is concentrated in hypothalamus as well as pituitary in males (Lloyd and Karavolas, 1975; Martini, 1982; Roselli et al., 1987). DHT suppresses LH secretion *in vivo* (Kao and Weisz, 1979; Ellis and Turek, 1980; Martini, 1982; Schanbacher, 1985) and *in vitro* (Tang and spies,

1975; Kamel and Krey, 1991). In castrated rams, DHT suppresses LH secretion by decreasing LH pulse frequency with little changes in LH pulse amplitude (Tilbrook et al., 1991). Furthermore, DHT fails to affect LH pulse amplitude in hypothalamo-pituitary disconnected wethers given frequent exogenous GnRH injection (Tilbrook et al., 1991). These observations suggest that DHT suppresses LH secretion by decreasing GnRH secretion at the hypothalamus. On the other hand, DHT decreases the responsiveness of pituitary to exogenous GnRH (D'Occhio et al., 1983; Tilbrook et al., 1991). Combined together, these findings suggest that DHT suppresses LH secretion at both the hypothalamus and pituitary in the ram.

Recently, Hileman et al. (1994) demonstrated that infusion of 5α-reductase inhibitor coincident with testosterone resulted in an increase in mean LH and LH pulse amplitude compared to testosterone infusion alone, representing that the inhibitory effects of testosterone on LH release in the ram are mediated, at least in part, by 5α-reduction of testosterone to DHT. However, studies on the rat using 5α-reductase inhibitor have demonstrated that the inhibitory effects of testosterone on LH secretion in vivo (Kao and Weisz, 1979) and on GnRH-induced LH release in vitro (Liang et al., 1984; Kamel and Krey, 1991) do not depend on its 5α-reduction.

CONCLUSION

Several lines of observations from simultaneous measurement of GnRH and LH release have demonstrated that pulsatile LH secretion in males is controlled primarily by the hypothalamus through concurrent secretion of GnRH.

This review has explored the role of gonadal steroids (testosterone, estradiol and DHT) on hypothalamo-pituitary axis in males. The role and effect of testosterone on GnRH secretion as well as synthesis may differ among species in males. In the male rat, there is controversy regarding to its role on GnRH secretion and synthesis. However, castration increased and testosterone replacement decreased GnRH pulse frequency in the male sheep and monkey. Although the hypothalamus appears to be the primary site for the action of testosterone negative feedback on LH secretion, several reports have demonstrated that testosterone may decrease LH release directly at the pituitary level by reducing the pituitary

responsiveness to GnRH. Up to date, however, the action of testosterone negative feedback at the pituitary is still debated.

In most previous studies, constant levels of testosterone were administered to examine the action of testosterone negative feedback on GnRH or LH secretion in males. Since testosterone is secreted in an episodic pattern, constant testosterone administration does not reflect the endogenous testosterone secretory patterns. Recently, more effective suppression of LH by a constant pattern of testosterone than a pulsatile pattern of testosterone has been reported. The mechanism underlying different effects of a constant versus a pulsatile pattern of testosterone on synthesis and secretion of gonadotropins remains to be understood.

Testosterone is either aromatized to estradiol or reduced to DHT in a variety of tissues. Treatment with aromatase inhibitor stimulates LH secretion in males and this effect is blocked by concurrent treatment with estradiol. Combined with the observations that estradiol prevents the increases in pituitary GnRH receptors after castration, those data suggest that estradiol may play an important role in the regulation of LH secretion in males at the level of hypothalamus and/or pituitary. DHT, the more potent androgen, suppresses LH secretion in males. Recent study have demonstrated that infusion of 5α-reductase inhibitor coincident with testosterone resulted in an increase in mean LH and LH pulse amplitude compared to testosterone infusion alone, supporting the hypothesis that the inhibitory effects of testosterone on LH release are mediated, at least in part, by 5α -reduction of testosterone to DHT.

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