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Negative feedback regulation of the secretion and actions of GnRH in male ruminants

A. J. Tilbrook[1] and I. J. Clarke[2]

[1]Department of Physiology, Monash University, and [2]Prince Henry's Institute of Medical Research, Clayton, Victoria, 3168, Australia

The roles of testicular hormones in the negative feedback regulation of the secretion and actions of GnRH in male domestic ruminants are reviewed, concentrating mainly on research conducted with rams. Testicular steroids have major feedback actions directly at the hypothalamus to inhibit the secretion of GnRH, although it is apparent that, under certain circumstances, the steroids also have actions directly at the pituitary gland. Further research is necessary to delineate these actions and to determine the contribution of testosterone and its primary metabolites, dihydrotestosterone and oestradiol, to negative feedback on the hypothalamo–pituitary unit. Since GnRH neurones do not possess receptors for steroids, testicular steroids must evoke other neuronal pathways to influence GnRH producing neurones. While the opioids may be important in this regard, it is necessary to determine which other neuronal pathways may also be involved to understand fully the mechanism of action of testicular steroids. It appears that the feedback regulation of the secretion of LH can be accounted for by testicular steroids, whereas the secretion of FSH is influenced by inhibin and steroids, and possibly the recently isolated proteins follistatin and activin. The actions of inhibin to suppress the secretion of FSH occur at the pituitary gland and not on the synthesis or secretion of GnRH. There is a complex interaction between testosterone and inhibin in the control of FSH secretion that results in synergistic effects during the non-breeding season but not during the breeding season. Activin has been shown to have FSH-stimulating properties and follistatin has been shown to have FSH-inhibiting properties, but it is unknown if these proteins play a physiological role in the feedback regulation of FSH in male domestic ruminants.

Introduction

The release of GnRH from the hypothalamus into the hypophysial portal blood is essential for the synthesis of LH and FSH from the anterior pituitary gland. This is demonstrated by suppression of the secretion of gonadotrophins after treatments that inhibit the actions of GnRH, such as immunization (see for example Lincoln and Fraser, 1979) or the use of GnRH analogues (see for example Fraser and Lincoln, 1980; Lincoln and Fraser, 1987). Measurement of GnRH in the hypophysial portal blood of rams has established that the secretion of GnRH is pulsatile and that there is a high degree of concordance between pulses of GnRH and LH (Caraty and Locatelli, 1988; Jackson *et al.*, 1991; Tilbrook *et al.*, 1991). In contrast to LH, the pattern of secretion of FSH in males is non-pulsatile (Fraser and Lincoln, 1980).

It is well established that the testes exert negative feedback on the hypothalamo–pituitary unit in males. This is clearly demonstrated by the rapid and significant rise in the plasma concentrations of LH and FSH in peripheral blood (see Tilbrook *et al.*, 1991 for references) and in the frequency of GnRH

pulses in hypophysial portal blood (Caraty and Locatelli, 1988) after castration. Negative feedback may occur at the hypothalamus to suppress the synthesis or secretion of GnRH or anterior pituitary gland to make it less sensitive to the actions of GnRH. The testes produce steroid hormones and the glycoprotein hormone inhibin, which may contribute to the negative feedback loop from the testis to the hypothalamo–pituitary unit. The negative feedback effects of the testicular steroids have been studied extensively but the contribution of inhibin to feedback regulation of gonadotrophins, and the interaction between inhibin and steroids, has received far less attention. Two recently isolated proteins, activin and follistatin, may also play a role in the regulation of the hypothalamo–pituitary axis. Here, we review the current understanding of the interactions between hormones in the feedback regulation of the secretion and actions of GnRH in males. Most research in this area has been with rams; therefore the review will concentrate largely on the hypothalamo–pituitary–testicular axis of rams.

Negative Feedback Regulation by Testicular Steroids

Administration of androgens and oestrogens to castrated rams (see Tilbrook *et al.*, 1991 for references) and bulls (Thompson *et al.*, 1984) has been shown to decrease the plasma concentrations of the gonadotrophins significantly, often to concentrations seen in intact males. Nevertheless, only recently has the extent to which the testicular steroids act at the hypothalamus or anterior pituitary gland been investigated in detail. Furthermore, the relative contribution of testosterone and its primary metabolites, dihydrotestosterone and oestradiol, to the negative feedback of gonadotrophins, and the mechanism by which steroids influence GnRH secretion are unknown.

Sites of action of testicular steroids

In rams, both androgens and oestrogens decrease the response of the pituitary to GnRH and decrease the frequency of pulses of LH, and it has been suggested that the testicular steroids feedback at both sites (see Tilbrook *et al.*, 1991 for references). The presence of steroid receptors in the hypothalamus (Pelletier and Caraty 1981; Glass *et al.*, 1984) and pituitary gland (Thieulant and Pelletier 1979, 1985; Glass *et al.*, 1984; Schanbacher *et al.*, 1984) strongly suggests that negative feedback by testicular steroids occurs at both the hypothalamus and pituitary. Other studies have suggested that the hypothalamus may be the principal site of negative feedback action of testicular steroids (Schanbacher, 1985a; Schanbacher and D'Occhio, 1984; Lincoln and Fraser, 1990). Nevertheless, the doses of testicular steroids used have not always been physiological. For example, steroidal treatments have often resulted in plasma concentrations of testosterone below and of 5α-dihydrotestosterone and oestradiol above physiological concentrations (Parrott and Davies 1979; D'Occhio *et al.*, 1983; Schanbacher 1984). Variable effects on the frequency and amplitude of LH pulses were observed in castrated rams given different concentrations of testosterone (D'Occhio *et al.*, 1982) which further complicates this issue. Furthermore, most previous studies have administered steroids in a manner that gives relatively constant concentrations in plasma, such as with the use of subcutaneous implants. This may not necessarily give meaningful physiological data because, in intact males, testosterone is secreted in pulses (Sanford *et al.*, 1974). Indeed, it was shown that constant infusion of testosterone is more effective than are pulses of testosterone in suppressing LH in castrated rams (Rhim *et al.*, 1993).

Conclusions have often been drawn from assessing the effects of steroid treatment on the pattern of secretion of LH in peripheral plasma. Decreases in the frequency of LH pulses are generally considered to be due to decreases in the frequency of GnRH pulses, indicating a hypothalamic site of action, whereas decreases in the amplitude of GnRH pulses suggest a reduced sensitivity of the pituitary to GnRH. However, this approach is inadequate, because a reduction in the amplitude of LH pulses may also represent a reduction in the amplitude of GnRH pulses due to hypothalamic actions by steroids. Furthermore, this approach does not consider the secretion of the other gonadotrophin, FSH.

Feedback at the hypothalamus. Direct measurement of GnRH in the hypophysial portal blood is necessary to determine whether testicular steroids have direct actions at the hypothalamus. Caraty and

Locatelli (1988) demonstrated that there was a clear and progressive increase in the frequency of GnRH pulses following castration of rams and, since they also found a good temporal relationship between GnRH and LH pulses, they concluded that this was due to a removal of negative feedback on GnRH secretion. More recently it was demonstrated that treatment of castrated rams with testosterone decreased the frequency of GnRH pulses (Jackson *et al.*, 1991; Tilbrook *et al.*, 1991). Other evidence for hypothalamic actions of testicular hormones has come from the use of GnRH antagonists. In rams treated with a GnRH antagonist, the frequency of LH pulses was suppressed, but following removal of the treatment there was a compensatory increase in the frequency of LH pulses (Lincoln and Fraser, 1987). The finding that extended blockade of LH secretion in castrated rams by a GnRH antagonist was not associated with a change in the pattern of secretion of GnRH in hypophysial portal blood (Caraty *et al.*, 1990) suggests that the compensatory increase in LH secretion following removal of GnRH antagonist treatment is due to an increase in the secretion of GnRH as a result of the decrease in circulating testicular hormones. When intact, cryptorchid and castrated rams were treated with a GnRH antagonist, the frequency of GnRH pulses in hypophysial portal blood was unaffected in the castrated rams and was increased in the intact rams (Caraty *et al.*, 1992). It was suggested that the results for the intact and cryptorchid rams reflected an escape from the negative feedback effects of testicular steroids on GnRH (Caraty *et al.*, 1992). Finally, in castrated rams treated with testosterone and administered a GnRH antagonist, there was no compensatory increase in LH secretion following removal of treatment compared with that of castrated rams (Lincoln and Fraser, 1990).

Feedback at the pituitary gland. Although measurement of GnRH in hypophysial portal blood allows assessment of actions at the hypothalamus, this approach does not provide information about direct actions at the pituitary gland. Feedback actions at the pituitary have mostly been assessed on the basis of a reduced release of LH following an injection of GnRH to castrated animals treated with steroids. This approach is inadequate to assess pituitary actions of hormones properly because the pituitary is still under the influence of hypothalamic releasing hormones, including GnRH. The hypothalamic influence on the anterior pituitary gland needs to be removed to define clearly the actions of testicular steroids on the pituitary. A model was developed in ewes that involved passive immunization against GnRH and administration of a GnRH agonist that was not recognized by the injected antibodies (Caraty *et al.*, 1984). When this approach was used in castrated rams perfused with testosterone, it was found that an injection of GnRH agonist was always able to induce a clear pulse of LH and it was concluded that testosterone had no feedback effects directly on the pituitary (Caraty *et al.*, 1992). Nevertheless, a single injection of GnRH does not reflect the pulsatile nature of GnRH secretion that occurs under physiological conditions. Therefore, to investigate feedback effects directly at the pituitary gland, we used the hypothalamo–pituitary disconnected sheep model (Clarke *et al.*, 1983) in castrated rams maintained on pulses of exogenous GnRH and treated with testicular hormones (Tilbrook *et al.*, 1991, 1993a). During the breeding season for rams, we found that treatment with testosterone, dihydrotestosterone or oestradiol had no significant effects on the plasma concentrations of LH or FSH or on the amplitude of LH pulses, suggesting that the feedback effects of testicular steroids at the pituitary are minimal (Tilbrook *et al.*, 1991). In contrast, during the non-breeding season, treatment of hypothalamo–pituitary disconnected castrated rams with testosterone significantly suppressed the plasma concentrations of FSH (Tilbrook *et al.*, 1993a) and LH (A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished). The discrepancies in our studies suggest that the feedback effects of testicular steroids at the pituitary vary with certain physiological conditions, such as the stage of the breeding season.

These findings imply that, when the hypothalamic inputs to the pituitary gland have been removed by hypothalamo–pituitary disconnection, the pituitary can still respond to seasonal stimuli with a consequent change in the sensitivity to feedback by testosterone. The sensitivity of the pituitary to negative feedback by testosterone appears to increase during the non-breeding season. We suggest that melatonin may be responsible for this seasonal regulation of the pituitary (Tilbrook *et al.*, 1993a), because high concentrations of melatonin receptors are present in the pars tuberalis (Morgan *et al.*, 1989) which remains with the pituitary after hypothalamo–pituitary disconnection (Clarke *et al.*, 1983). Further research is required to test this hypothesis.

Relative contribution of testosterone metabolites to negative feedback

Although testosterone is the steroid produced in the largest quantity by the testes in most species, the testes also produce oestrogens and other androgens. Furthermore, testosterone is reduced to 5α-dihydrotestosterone and aromatized to oestradiol in target tissues. Although each of these steroids has been shown to suppress the secretion of the gonadotrophins in castrated males, the relevance and the degree of interaction between androgens and oestrogens has not been clearly delineated and their sites of action have not been determined. In castrated rams treated with an aromatase inhibitor, the ability of testosterone to inhibit the secretion of LH was compromised (Schanbacher, 1984) and the secretion of LH was high in intact rams treated with an antibody to oestradiol (Sanford, 1987), suggesting that oestradiol mediates, at least in part, the inhibitory effects of testosterone on the secretion of LH. Infusion of castrated rams with a 5α-reductase inhibitor provided evidence that conversion of testosterone to dihydrotestosterone also represents an important step in the negative feedback regulation of gonadotrophins (Hileman *et al.*, 1994). Infusion of the reductase inhibitor in conjunction with testosterone increased the concentrations of LH above those in animals treated with testosterone, but not to concentrations in animals treated with the reductase inhibitor alone (Hileman *et al.*, 1994). Nevertheless, formal and systematic comparisons of the effects of each of these steroids, using physiological treatments, have not been conducted. During the breeding season, castrated rams were treated with testosterone, dihydrotestosterone and oestradiol and each of the steroids could suppress secretion of LH and FSH (Tilbrook *et al.*, 1991). Unfortunately, it was not possible to conclude anything about the relative importance of each of the steroids in the negative feedback effects on the hypothalamo–pituitary unit because, although the dose of testosterone used was physiological, the dose of dihydrotestosterone was probably supraphysiological and plasma concentrations of oestradiol were not measured. Nonetheless, the dose of oestradiol was chosen on the basis that it gave physiological concentrations of oestradiol in ewes. The finding that none of these testicular steroids influenced the secretion of LH or FSH in hypothalamo–pituitary disconnected castrated rams during the breeding season provides further evidence that feedback effects of steroids at the pituitary are minimal (Tilbrook *et al.*, 1991). Nevertheless, studies of this nature have not been undertaken during the non-breeding season and there are no published reports of a comparison of the effects of androgens and oestrogens on the concentrations of GnRH in hypophysial portal blood of males.

Mode of action of testicular steroids at the hypothalamus

Although testicular steroids have major feedback effects directly on the hypothalamus, the means by which these steroids influence the activity of GnRH producing cells is unknown. GnRH neurones do not possess receptors for steroids (Shivers *et al.*, 1983; Watson *et al.*, 1992; Huang and Harlan, 1993) which means that the negative feedback effects of the testicular steroids must be mediated through other neuronal cell types that influence GnRH neurones. There are many afferents to GnRH neurones (Fig. 1) which have the potential to receive and transmit steroidal feedback signals (for review see Clarke, 1994) and the effects of these may be manifest by actions directly on the cell bodies or on the terminals of GnRH neurones (Clarke, 1994). It is difficult to ascertain which neural systems are relevant to steroid negative feedback at any particular time and, although there has been much work in females to investigate the role of neurotransmitters in the brain regulation of the secretion of GnRH (see Thiery and Martin 1991 for review), little is known about the role of various neural pathways in the control of GnRH secretion or synthesis in males. It may be misleading to assume that the brain control of GnRH secretion in females and males is similar because the neuroanatomical and neuroendocrine organization of the reproductive processes of the two sexes is different and, therefore, is likely to rely on different neuronal systems under various circumstances. An obvious major difference in the reproductive endocrinology of males and females is the presence of the LH surge centre in the female brain which develops owing to a lack of testosterone during ontogeny (see Clarke, 1992 for review). In sheep, it was demonstrated that there is clear sexual differentiation of the neuroendocrine mechanisms that regulate seasonal changes of the secretion of LH (Lubbers and Jackson, 1993). Furthermore, whereas the dopaminergic system, operating via D_2 receptors, is involved in the negative feedback effects of oestradiol in ewes during the

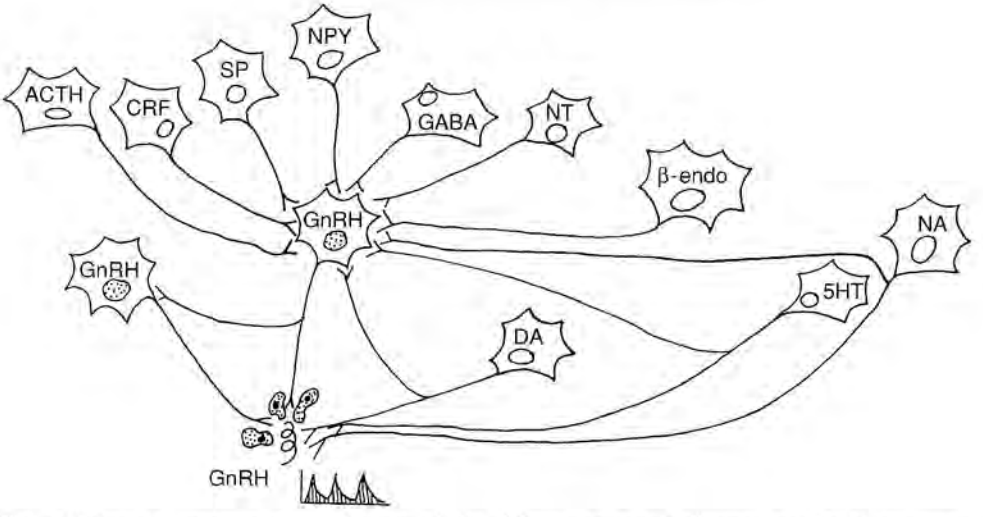

Fig. 1. Schematic representation of some of the different neuronal afferents on GnRH neurones. GnRH: gonadotrophin releasing hormone; ACTH: adrenocorticotrophic hormone; CRF: corticotrophic releasing factor; SP: substance P, NPY: neuropeptide Y; GABA: gamma aminobutyric acid; NT: neurotensin; β-endo: β-endorphin; 5HT: 5-hydroxytryptamine; NA: noradrenaline; DA: dopamine.

non-breeding season, this system does not appear to be involved in the negative feedback effects of testosterone in rams (Tilbrook and Clarke, 1992). Recently, we found that injections of neurotensin or neuropeptide Y into the medial preoptic area of the hypothalamus or the third ventricle of castrated rams did not affect the secretion of LH in the presence or absence of treatment with testosterone (A. J. Tilbrook, G. L. Jackson and I. J. Clarke, unpublished), suggesting that these neurotransmitters do not play a major role in the mediation of negative feedback by testicular steroids in rams.

Research in rams has suggested that opioid peptides may play a role in relaying some of the negative feedback effects of testosterone. The frequency of LH pulses in rams was increased following treatment with the opioid antagonist naloxone, whereas the opioid agonist morphine had the opposite effect (Ebling and Lincoln, 1985; Schanbacher, 1985a). These effects in castrated rams were increased when the rams were treated with testosterone (Schanbacher, 1985a; Lincoln *et al.*, 1987) or oestradiol (Schanbacher, 1985b). Caraty *et al.* (1987) measured GnRH in the hypophysial portal blood of intact, short-term castrated and long-term castrated rams following treatment with naloxone and found that GnRH release was increased in all rams but the response was far greater in intact rams followed by short-term castrated rams and then in long-term castrated rams. They suggested that the inhibitory effects of opioids on the secretion of LH are diminished during escape from castration (Caraty *et al.*, 1987). These findings support the contention that opiods are involved in the effects of steroids on the secretion of GnRH. Furthermore, it appears that these effects of opioids may be confined to the breeding season in rams. Naloxone induced a clear dose–response increase in plasma concentrations of LH in intact rams and castrated rams treated with testosterone under stimulatory photoperiods but, during inhibitory photoperiods, even the highest dose of naloxone failed to increase LH in intact rams, and caused a small increase in castrated rams treated with testosterone (Ebling and Lincoln, 1985).

Negative Feedback Regulation by Inhibin

It is apparent that testicular steroids do not fully account for the negative feedback regulation of FSH in male domestic ruminants. For example, when castrated rams were treated with a physiological dose of testosterone for a week there was a non-significant reduction in plasma concentrations of FSH of only 15%, whereas LH was reduced by 80% to concentrations similar to those found in intact rams (Tilbrook

et al., 1991). This finding suggests that another testicular product, such as inhibin, is important for the negative feedback regulation of FSH. The accepted definition of inhibin is that it is a glycoprotein hormone consisting of two dissimilar subunits, α and β, joined by disulfide bonds, which inhibits the production or secretion by the pituitary gland of the gonadotrophins, preferentially FSH (Burger and Igarashi, 1988). Studies in rams and bulls that have been administered preparations of inhibin, or have been immunized against inhibin, strongly suggest that inhibin plays a major role in the feedback regulation of FSH (see Tilbrook *et al.*, 1992 for review).

The most compelling evidence that inhibin is a major feedback regulator of FSH secretion has come from studies in which we administered purified inhibin to castrated rams. A single i.v. injection of 50 μg human recombinant inhibin A significantly suppressed plasma concentrations of FSH (Tilbrook *et al.*, 1993a). Furthermore, treatment of castrated rams that had undergone hypothalamo–pituitary disconnection and were maintained on pulses of GnRH suppressed the plasma concentrations of FSH, indicating that inhibin has feedback actions directly at the pituitary gland (Tilbrook *et al.*, 1993a). Infusion of castrated rams for 12 h with a dose of human recombinant inhibin A that resulted in physiological concentrations of inhibin in the plasma suppressed plasma concentrations of FSH to values similar to those observed in intact rams (Tilbrook *et al.*, 1993b). This treatment had no effect on the plasma concentrations, pulse amplitude or pulse frequency of LH (Tilbrook *et al.*, 1993b). Collectively, these results indicate that inhibin is a major and selective feedback regulator of the secretion of FSH in rams and that its actions occur at the pituitary gland and not on the synthesis or secretion of GnRH.

Interaction between Inhibin and Testicular Steroids in the Feedback Regulation of FSH

While inhibin clearly plays a major, if not the major, role in the regulation of FSH secretion in male domestic animals, the testicular steroids are obviously also involved and it is difficult to assess the relative importance of each in the feedback regulation of FSH. Steroids or inhibin preparations have most often been administered in isolation, which gives misleading results because these hormones are never alone in the bloodstream. We administered human recombinant inhibin A and testosterone alone and in combination to hypothalamo–pituitary disconnected and hypothalamo–pituitary intact castrated rams and found that both hormones had direct actions on the pituitary to suppress plasma concentrations of FSH (Tilbrook *et al.*, 1993a; A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished). Furthermore, during the non-breeding season, the suppressive effects of human recombinant inhibin A were greater when the hypothalamo–pituitary disconnected (Tilbrook *et al.*, 1993a; A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished) or hypothalamo–pituitary intact (A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished) castrated rams were being treated with testosterone than in the absence of testosterone, suggesting a synergistic interaction between inhibin and testosterone in the control of FSH secretion. This synergistic interaction was not apparent during the breeding season, although both inhibin and testosterone could suppress plasma concentrations of FSH (A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished). This finding further illustrates how the feedback system of males may vary with different physiological states. Further investigation is required using an experimental model that provides physiological treatments of both steroids and of inhibin.

Activin and Follistatin in the Regulation of the Secretion and Actions of GnRH

A proper understanding of the feedback regulation of the secretion of gonadotrophins in males must consider two other classes of proteins, the activins and follistatin, which may have FSH-stimulating or -inhibiting properties (see de Paolo *et al.*, 1991 for review). The activins represent disulfide-linked dimers of the β-subunits of inhibin, termed activin A ($\beta_A\beta_A$ dimer) and activin AB ($\beta_A\beta_B$ dimer), which were first isolated from pig follicular fluid and shown to stimulate the secretion of FSH *in vitro* (see de Paolo *et al.*, 1991 for review). It has not been determined whether the activins play a role in the regulation of the secretion of gonadotrophins in male domestic ruminants.

Follistatin (Ueno *et al.*, 1987) or FSH-suppressing protein (FSP: Robertson *et al.*, 1987) is a single protein, structurally distinct from inhibin and was isolated from porcine (Ueno *et al.*, 1987) and bovine

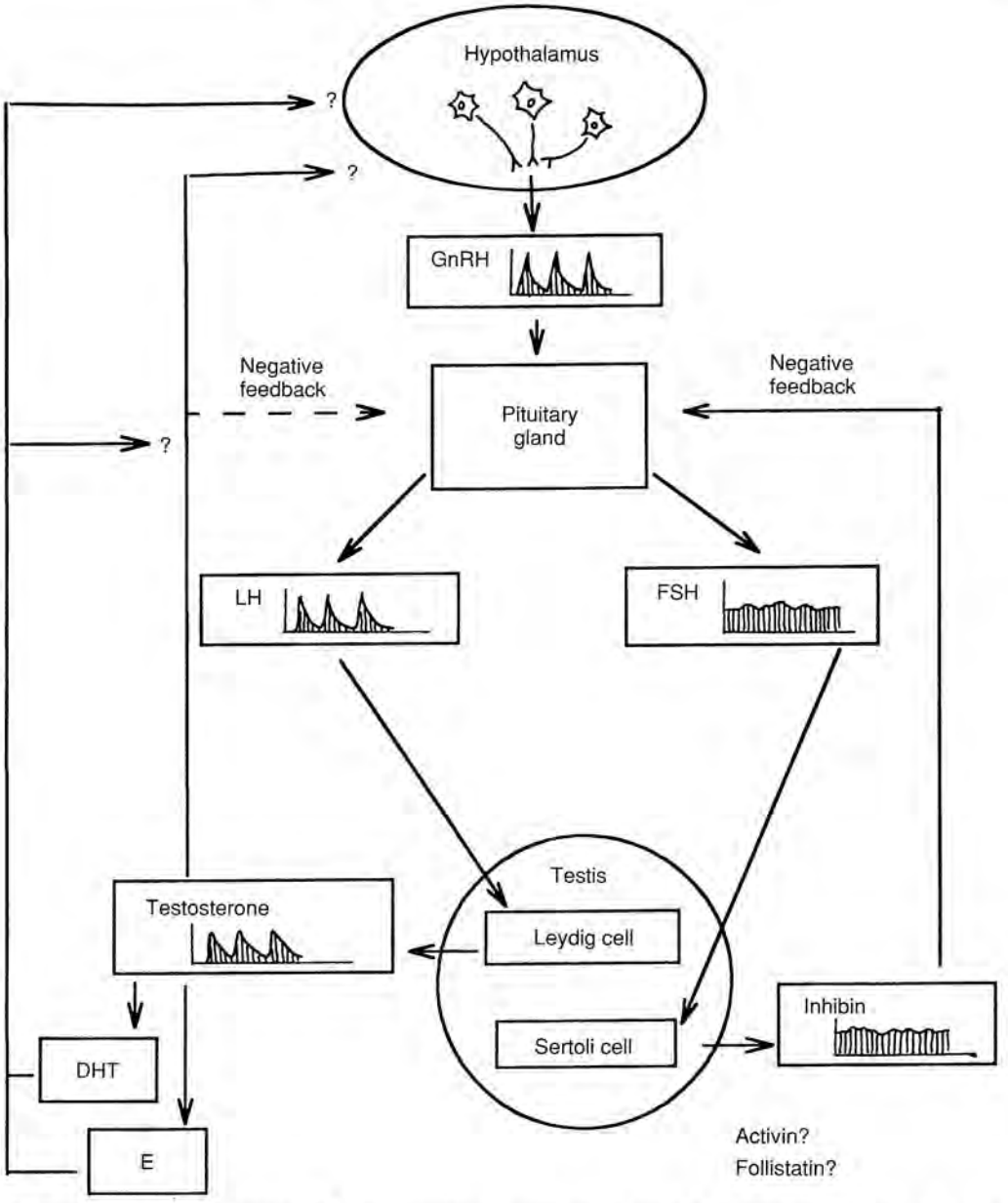

Fig. 2. Schematic representation of the hormonal interactions of the hypothalamo–pituitary–testicular axis of male domestic ruminants. This figure illustrates that the pattern of secretion of GnRH, LH and testosterone is pulsatile, whereas FSH and inhibin show non-pulsatile patterns of secretion. Solid lines indicate major sites of action and a broken line indicates a secondary site of action. Testosterone is converted by 5α-reduction to 5α-dihydrotestosterone (DHT) and by aromatization to oestradiol (E). Proposed mechanisms are indicated by a question mark (?). Activin and follistatin have FSH-stimulatory and inhibitory actions, respectively, but their sites and mechanisms of action, and physiological importance in the feedback regulation of FSH are unknown. Nevertheless, there is evidence to suggest that the action of follistatin to suppress FSH may be due to its capacity to bind and neutralize activin in the pituitary.

(Robertson *et al.*, 1987) follicular fluid. Follistatin was demonstrated to suppress FSH *in vitro* with a potency 10–30% of purified inhibin (see for example Ueno *et al.*, 1987; Robertson *et al.*, 1987). Follistatin has also been shown to have the capacity to bind activin (Nakamura *et al.*, 1990) and there is evidence to suggest that the actions of follistatin in suppressing FSH may be due to its capacity to bind, and neutralize, activin in the pituitary (Corrigan *et al.*, 1991) although, as for activin, it is unknown whether follistatin has a physiological role in the regulation of FSH secretion in male domestic ruminants. Recently, we measured circulating concentrations of follistatin in rams and found that, although the testis contains follistatin, it is not the major source of circulating follistatin (Tilbrook *et al.*, 1993c). We infused human recombinant follistatin into castrated rams to address the issue of whether follistatin is likely to regulate the secretion of gonadotrophins (A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished). The dose and treatment regimen of human recombinant follistatin was identical to the treatment of human recombinant inhibin that yielded physiological concentrations of inhibin and FSH in castrated rams (Tilbrook *et al.*, 1993b). Human recombinant follistatin significantly suppressed plasma concentrations of FSH, but the suppression was about 2.5 times lower than that achieved with human recombinant inhibin, suggesting that follistatin plays a role in the negative feedback control of FSH secretion in rams, but it is probably a less potent suppressor of FSH secretion than is inhibin. The extent to which follistatin may interact with inhibin and testicular steroids in the feedback regulation of FSH is unknown. None of the parameters of LH secretion was influenced by treatment with human recombinant follistatin (A. J. Tilbrook, D. M. de Kretser and I. J. Clarke, unpublished) indicating that, like inhibin, this protein is unlikely to have direct effects on GnRH synthesis or secretion.

Conclusions

The hypothalamo–pituitary unit in males is under the feedback regulation of both testicular steroids and inhibin (Fig. 2). Although it is tempting to conclude that the predominant site of action of testicular steroids is the hypothalamus, these hormones clearly have actions at the pituitary under certain circumstances. Further research is necessary to establish the extent to which steroids have hypothalamic and pituitary actions under particular physiological conditions, such as with the stage of the breeding season. Furthermore, the extent to which negative feedback actions of testicular steroids are due to testosterone or its primary metabolites is not apparent and the neural pathways through which testicular steroids evoke their negative feedback effects at the hypothalamus are unknown, although opioids appear to play a role. The secretion of LH appears to be accounted for totally by the feedback actions of steroids, whereas FSH is controlled by inhibin and steroids, and possibly follistatin. It is unknown whether activin and follistatin play significant roles in the regulation of the secretion and actions of GnRH in male domestic ruminants.

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