Is there an FSH-releasing factor?

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A thorough understanding of the factors that regulate the secretion of FSH is critical for the development of efficient methods for fertility regulation. The purpose of this review is to evaluate what is currently known about the existence of FSH-releasing factor(s). It considers the obstacles encountered in understanding the control of FSH secretion, current knowledge of the nature of FSH secretion and the mechanisms involved in controlling FSH secretion, the arguments that have been posed against the need for an FSH-releasing factor and finally the evidence available to date supporting the existence of FSH-releasing factor(s).

Follicle-stimulating hormone (FSH) is a key reproductive hormone involved in the regulation of follicular development. In comparison with luteinizing hormone (LH), progress in understanding the secretory nature of FSH and its control is limited. Part of the problem in assessing the secretory dynamics of FSH in the periphery has stemmed from its long half-life and molecular heterogeneity (for review, see Ulloa-Aguirre *et al.*, 1995). The inability of existing assays to recognize all circulating isoforms of FSH may also have been a contributing factor. In addition, because gonadotrophin α -subunit is secreted in a pulsatile manner (Hall *et al.*, 1990), and FSH and LH share a common α -subunit, it is unclear whether the episodic pattern of FSH release, when observed, is a reflection of gonadotrophin α -subunit or LH crossreactivity. In spite of these caveats, several advances have been made in our understanding of the control of FSH secretion.

Current understanding of the nature of FSH secretion

Basal and episodic modes of FSH release

In contrast to the absolute dependence of the LH secretory system on GnRH (LHRH) pulsatility, FSH secretion appears to be regulated by a dual mechanism, one mechanism controlling a basal (constitutive) and the other a pulsatile (regulated) component. That a substantial portion of circulating FSH reflects constitutive secretion and is not reliant on immediate stimulus secretion coupling is supported by the following findings: (1) continued release of FSH for prolonged periods in hypothalamic disconnected sheep (Clarke *et al.*, 1983); (2) continued release of FSH in hypophysectomized rats bearing pituitary transplants

under the kidney capsule (DePaolo, 1991); and (3) continued release of FSH by long-term pituitary cultures (Sheridan *et al.*, 1979). This component of FSH release appears to be dependent on the availability of translatable FSH β mRNA (Clarke *et al.*, 1993; Muyan *et al.*, 1994; Farnworth, 1995).

What is the evidence available in support of the existence of an episodic component of FSH secretion? Barring a few studies, in which distinct patterns of episodic FSH have been surmised from peripheral measurements (for example, in rats: Culler and Negro-Vilar, 1987), pulses of FSH measured at the periphery in general are not as discrete as pulses of LH (for example, in sheep: Wallace and McNeilly, 1986; and humans: Gross *et al.*, 1987). In most studies, investigators resort to statistical approaches to deconvolute FSH pulses. This obscurity in defining FSH secretory patterns can be overcome by assessing secretory patterns of FSH at a site close to release in the hypophyseal portal vasculature.

Hypophyseal portal blood as a source to assess FSH secretory dynamics

Continuous withdrawal of hypophyseal portal blood, a method pioneered by Clarke *et al.* (1983), in addition to providing a direct and detailed understanding of the pulsatile secretion of GnRH in sheep, provides an excellent opportunity to determine secretory patterns of gonadotrophins near their sites of release (Midgley *et al.*, 1997). During hypophyseal portal collection, portal vessels are lesioned at the surface of the pituitary. Gonadotrophins may enter the portal circulation via any of three routes: (1) from gonadotrophs located in the pars tuberalis surrounding the stalk of the adenohypophysis; (2) from retrograde flow through hypophyseal portal vessels; and (3) from drainage of sinusoids lesioned in the pituitary.

Comparison of LH patterns in simultaneous samples of peripheral and hypophyseal portal blood of ovariectomized ewes demonstrates the outstanding resolution that

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Fig. 1. Patterns of GnRH (red), hypophyseal portal and jugular (peripheral) LH (a) and FSH (b) (blue) from an ovariectomized ewe during anoestrus (V. Padmanabhan, G. D. Dahl, N. P. Evans, F. J. Karsch and J. Van Cleeff, unpublished). Red asterisks indicate GnRH-associated pulses and blue asterisks the non-GnRH-associated pulses. Note: (1) the discreteness and magnitude of LH in hypophyseal portal blood; (2) the one-to-one relationship of both hypophyseal portal and peripheral LH with GnRH; (3) the dynamic pattern of FSH in hypophyseal portal but not peripheral secretion; and (4) the GnRH-associated and non-GnRH-associated pulses of FSH. Differences in the thresholds of LH and FSH in peripheral and hypophyseal portal circulation appear to reflect the recirculating concentrations of LH and FSH (yellow area) and is the sum of their secretion, clearance and peripheral metabolism.

is achieved in characterizing secretory patterns of pituitary hormones using this approach (Fig. 1a). Because blood is collected close to the site of release, and masking of the true secretory patterns by recirculating hormones is minimized by the massive secretory output, LH is concentrated several-fold in hypophyseal portal compared with peripheral blood, where its pattern of secretion is more discrete than in the peripheral circulation. Furthermore, LH pulse patterns in hypophyseal portal, as in peripheral, circulation follow GnRH patterns closely, with a virtual one-to-one relationship between the two hormones. The discrete LH pattern and its close relationship to GnRH indicate that the dynamics of pituitary hormones observed in pituitary portal

blood reflect the secretory dynamics and not leakage from damaged pituitary cells.

Characterization of FSH patterns in hypophyseal portal blood reveals a dynamically changing pattern of FSH that is hard to surmise from peripheral measurements (Fig. 1b). In sheep, studies using the experimental approach described above and a well-characterized FSH assay that crossreacts minimally with alpha subunit (< 0.3%) have demonstrated unequivocally that there is an episodic component of FSH secretion (Padmanabhan *et al.*, 1997). This finding corroborates what has been surmised previously on the basis of peripheral measurements of FSH. What is the nature of this episodic FSH secretion, and can it be

Fig. 2. Pulse concordance relationships of hypophyseal portal (secreted; P) and jugular (peripheral; J) FSH and GnRH in ovariectomized ewes (red; from Padmanabhan *et al.*, 1997). Although pulses in peripheral circulation are not as discrete and visually identifiable as they are in hypophyseal portal circulation, peripheral pulses identified using pulse detection algorithms were used to assess these relationships. When the percentage of GnRH with corresponding FSH pulses was computed (GnRH as the denominator), the concordance estimate was 93% for portal FSH and 73% for peripheral FSH (a). The concordance between hypophyseal portal and peripheral FSH was 70% (b). In contrast, when the percentage of FSH pulses showing an association with GnRH (FSH as the denominator) was computed (note there are more pulses of FSH than of GnRH), only 68% of FSH pulses were found to be associated with GnRH owing to the existence of non-GnRH-associated pulses of FSH (32%) (c). For comparison, the corresponding relations of LH are provided (blue). Irrespective of whether GnRH or LH was used as the denominator, there is a 100% concordance between GnRH, hypophyseal portal LH, and peripheral LH, demonstrating that GnRH is the sole regulator of LH pulsatility. (Redrawn from Padmanabhan *et al.*, 1997.)

accounted for completely by the changes in GnRH secretion?

Evidence for the existence of GnRH-independent FSH pulses

Key to our understanding of whether GnRH can account for the pulsatile control of FSH secretion is the determination of whether all identified pulses of FSH are concurrent with GnRH pulses. cursory assessment of the data shown (Fig. 1) reveals the existence of both GnRH-associated (red asterisks) and non-GnRH-associated (blue asterisks) pulses of FSH in hypophyseal portal blood. The up slope of some of the GnRH-associated FSH pulses in some instances precedes the start of GnRH pulses. This finding contrasts with the pattern of LH in which increases in LH are coincident with, or follow, GnRH increases. As the

blood passing from the hypophyseal portal circulation is not returned (washout can lead to fast disappearance times), increases in FSH concentrations provide evidence for incremental, active secretion. Does this mean that there is a second component even in the GnRH-associated pulses? Studies in ovariectomized sheep have established that almost all (93%) of the GnRH pulses show correspondence with FSH pulses (Padmanabhan *et al.*, 1997) (Fig. 2). These GnRH-associated pulses accounted for only two-thirds of the total FSH pulses observed. Thus, one-third of the total FSH pulses remained unaccounted for by GnRH.

Further evidence for a GnRH-independent, pulsatile FSH component comes from the studies of Culler and Negro-Vilar (1987) demonstrating that combined administration of GnRH antiserum and a GnRH antagonist to castrated male rats abolishes LH pulsatility completely without abolishing FSH pulsatility. Pau *et al.* (1991) corroborated

Fig. 3. Schematic representation of the three proposed mechanisms by which a single-releasing hormone (GnRH) can differentially regulate FSH secretion. Option 1 (a): changes in GnRH frequency, with high frequency GnRH pulses favouring the overall concentration of LH over FSH and low frequency GnRH favouring FSH over LH. Option 2 (b): amplitude modulation, with low amplitude GnRH selectively releasing FSH over LH. Option 3 (c): changes in response time, with FSH taking longer to respond than LH. Options 1 and 3 do not reasonably explain the occurrence of additional non-GnRH-associated pulses of FSH. In addition, since LH is more sensitive than FSH to GnRH (Normolle *et al.*, 1997), option 2 does not explain the occurrence of non-GnRH-associated pulses of FSH.

these findings in rabbits by administering phentolamine (an α -adrenergic antagonist) intravenously to disrupt the generation of medial basal hypothalamic GnRH pulses, or GnRH antiserum intra-hypothalamically to immunoneutralize endogenous GnRH pulses. Both treatments selectively suppressed LH pulsatility but not FSH pulsatility. What other lines of evidence support the presence of additional control mechanisms for FSH?

Differential regulation of LH and FSH

Although GnRH has been proven unquestionably to induce the release of both LH and FSH (Schally *et al.*, 1971), the patterns of LH and FSH have been shown to diverge often. For instance, disruption of α -adrenergic neurotransmission results in a rapid decrease in LH but not FSH pulse frequency (Dobson and Ward, 1977; Chappel *et al.*, 1984). Catecholamine turnover within specific hypothalamic nuclei accompanies alterations in LH but not FSH secretion (Barraclough and Wise, 1982). Dissimilarities in the

responses of LH and FSH to electrochemical stimulation (Chappel and Barraclough, 1976), lesion (Bishop *et al.*, 1972), gonadal steroids (Kalra, 1976; Strobl and Levine, 1988; Gharib *et al.*, 1990), gonadal proteins (Gharib *et al.*, 1990; Baird *et al.*, 1991) and GnRH antagonists (Kartun and Schwartz, 1987; Hall *et al.*, 1990; Normolle *et al.*, 1997) have also been observed. Both LH and FSH are often produced in the same gonadotrophs and may even be stored in the same secretory granules within gonadotrophs (Childs, 1986). If GnRH is the only hypothalamic regulator of FSH, what mechanisms might explain the discordance in the episodic pattern of LH and FSH secretion?

Mechanisms proposed to explain why differential release of LH and FSH does not require the presence of an FSH-releasing factor

GnRH input pattern

Several mechanisms have been proposed to explain the

apparent paradox of differential LH and FSH release (Fig. 3). First, an alteration in GnRH pulse frequency has been recognized as a mechanism by which a single releasing hormone can regulate LH and FSH differentially, slower frequencies favouring FSH release and faster frequencies favouring LH release (Wildt *et al.*, 1981; Clarke *et al.*, 1984; Marshall *et al.*, 1992) (Fig. 3a). Although the overall threshold of FSH secretion can certainly be mediated by changes in GnRH pulse frequency, this mechanism does not help explain the existence of the non-GnRH-associated pulses of FSH discussed above.

Second, differential sensitivity of the subpopulation of gonadotrophs that secrete LH and FSH (Denef *et al.*, 1978; Childs 1985) or threshold differences in the response to GnRH (amplitude modulation) with small GnRH infusions favouring FSH secretion (Wise *et al.*, 1979; Wu *et al.*, 1987) have been suggested as alternative means by which LH and FSH can be regulated differentially (Fig. 3b). Dose-response studies *in vitro* (Normolle *et al.*, 1997) show LH and not FSH to be more sensitive to GnRH. Studies comparing the secretory responses of FSH and LH to different doses of GnRH are lacking *in vivo*.

Third, different response times of LH and FSH to GnRH (Schwartz *et al.*, 1985) may be a means by which non-GnRH-associated pulses can originate (Fig. 3c). However, this mechanism does not explain the disproportionately larger number of FSH pulses (compared with GnRH-LH pulses) that are often encountered. Furthermore, exogenous administration of pulsatile GnRH, at least in studies *in vitro*, leads to synchronous and accountable increases in LH and FSH (Weiss *et al.*, 1990).

If any of the above three arguments are a valid explanation for the non-GnRH-associated pulses of FSH, FSH pulsatility should be abolished after the blockade of GnRH action. However, FSH pulsatility is not abolished since episodic FSH secretion has been shown to persist even after elimination of endogenous GnRH activity (Culler and Negro-Vilar, 1987; Kartun and Schwartz, 1987; Hall *et al.*, 1990). These findings provide the strongest evidence in support of the existence of an additional non-GnRH-dependent mechanism regulating FSH pulsatility.

Metabolic clearance versus endocrine milieu

Differences in metabolic clearance rates may explain the apparent differences in LH and FSH release. Such differences are well documented (for review, see Ulloa-Aguirre *et al.*, 1995) and certainly contribute to changes in baseline concentrations of circulating LH and FSH. Although pulses of FSH can be obscured by slow clearance, differences in the clearance rates of LH and FSH cannot explain the asynchronous initiation of FSH pulses (Culler and Negro-Vilar, 1987; Pau *et al.*, 1991; Padmanabhan *et al.*, 1997).

Differences in the circulating endocrine milieu (gonadal steroids and peptides) may also have differential effects on the release patterns of LH and FSH (Savoy-Moore and Schwartz, 1980; McNeilly 1988; Baird *et al.*, 1991).

Oestradiol is a major negative feedback regulator of both LH and FSH secretion. Differential effects of oestradiol on LH and FSH appear to be mediated both via alteration of hypothalamic GnRH secretion (Marshall *et al.*, 1992; Karsch and Evans, 1996) and via direct effects at the pituitary. Direct effects of oestradiol appear to be mediated mainly at transcription and translation (for review, see Gharib *et al.*, 1990; Brown and McNeilly, 1999). Oestradiol has also been shown to cause the movement of granules containing LH to the plasma membrane in ovine gonadotrophs without altering the distribution of granules containing FSH (Thomas and Clarke, 1997). Evidence that gonadally derived inhibin is involved in selectively suppressing overall FSH production (McNeilly, 1988; Ying 1988; Baird *et al.*, 1991) is also very strong. Although the effects of these long-range endocrine regulators in altering the baseline concentration of FSH is uncontested, they do not provide an explanation for the occurrence of discordant LH and FSH pulses in gonadectomized animals (Culler and Negro-Vilar, 1987; Pau *et al.*, 1991; Padmanabhan *et al.*, 1997).

None of the arguments posed thus far provide sufficient explanation for the asynchronous episodic LH and FSH release. Is the non-GnRH-associated episodic FSH release the consequence of a changing local pituitary environment having an impact on the constitutive secretagogue-independent mode of FSH secretion or is a separate neural trigger required to explain fully the differential control of LH and FSH secretion?

Pituitary control

An inherent pituitary-driven mechanism (Chappel, 1985) may explain the non-GnRH-associated episodic FSH release. This possibility has not been fully tested *in vivo* in GnRH-deficient models using frequent sampling paradigms, but the absence of a pulsatile pattern of FSH release from pituitary tissue *in vitro* (Turgeon and Waring, 1982) is not consistent with this hypothesis. Another possibility is that the non-GnRH-associated releases of FSH are caused by changes in locally produced inhibin, activin or follistatin (Fig. 4). This hypothesis is supported by the fact that activins, inhibins and follistatins are produced in the anterior pituitary (DePaolo *et al.*, 1991; Mather *et al.*, 1992).

FSH biosynthesis and release are inhibited by inhibins and follistatins and stimulated by activins (for review, see Ying, 1988; DePaolo *et al.*, 1991; Mather *et al.*, 1992). Activin is more potent than GnRH at regulating FSH β mRNA (Weiss *et al.*, 1992) and the effects of GnRH appear to be mediated via alterations in local activin availability (Besecke *et al.*, 1996). Activins, inhibins and follistatins were thought originally to be produced only at the gonads and to act in an endocrine manner to modulate FSH secretion. Although the evidence supporting an endocrine role for inhibin is strong (Baird *et al.*, 1991), some studies have questioned an endocrine role for follistatin and activin (Muttukrishna *et al.*, 1996; McConnel *et al.*, 1998). Most, if not all, activin in plasma appears to be bound by follis-

Fig. 4. Schematic representation of the local pituitary loop involving activin, inhibin and follistatin and their impact on FSH secretion. This model predicts that increases in pituitary activin or decreases in inhibin and/or follistatin would increase FSH secretion. In contrast, decreases in activin or increases in follistatin–inhibin would lower FSH secretion.

tatin and to be biologically inert. Therefore, the actions of activin and follistatin in regulating FSH release are believed to be mediated via autocrine–paracrine mechanisms at the pituitary (Fig. 4).

The functional overlap among these regulators must be taken into account to understand how they modulate FSH production and secretion (Fig. 4). Inhibins and activins are functional opposites (Ying, 1988). Although there is evidence that there are specific, separate receptors for inhibin (Draper *et al.*, 1998; Hertan *et al.*, 1999), inhibin is able to bind to activin receptors, albeit with lower affinity (Mathews and Vale, 1991; Martens *et al.*, 1997), and to antagonize the action of activin (Weiss *et al.*, 1993). Follistatin, a binding protein of activin and inhibin, neutralizes activin but not inhibin action (Robertson, 1992). Therefore, the relative proportion of these three regulators is an important consideration in determining their overall impact on FSH release. An increase in activin or a decrease in inhibin and follistatin production at the anterior pituitary would increase FSH production and secretion. Conversely, a decrease in activin or an increase in inhibin and follistatin would decrease FSH production and secretion.

Regulation of pituitary factors controlling FSH synthesis and release

The factors involved in regulating anterior pituitary activin, inhibin and follistatin to control FSH synthesis and release in different physiological situations may be both hypothalamic and endocrine. For example, there is a reciprocal

relationship between the expression of FSHβ and mRNAs encoding follistatin in response to different patterns of GnRH treatments in perfused, rat pituitary cells (Kirk *et al.*, 1994; Besecke *et al.*, 1996). Infrequent pulses of GnRH stimulate only expression of FSHβ mRNA, whereas higher frequency pulses increase expression of follistatin mRNA (Kirk *et al.*, 1994; Besecke *et al.*, 1996). However, it is slow frequency GnRH pulses that selectively increase circulating concentrations of FSH *in vivo* (Clarke *et al.*, 1984; Wildt *et al.*, 1981; Marshall *et al.*, 1992). Dalkin *et al.* (1999) have shown that low amplitude GnRH pulses also increase expression of activin βB mRNA. Collectively, these findings indicate that the local pituitary milieu is the means by which changes in GnRH input lead to differential release patterns of LH and FSH. However, the suggested role of activin, inhibin and follistatin in the local regulation of pituitary FSH is based on observations of changes in the steady states of their mRNAs and are yet to be validated at the protein level.

Although the changes in baseline concentration of FSH release can be explained by changes in regulatory factors produced in the anterior pituitary, it is unclear whether such changes can also explain the episodic pattern of FSH release. Regulatory factors must also undergo correlated changes to account for the non-GnRH-dependent episodic mode of FSH secretion. At the periphery, there is evidence that inhibin is secreted in a pulsatile manner (McNeilly and Baird, 1989; Lockwood *et al.*, 1998). Can the pulsatile mode of peripheral inhibin secretion account for the non-GnRH-dependent episodic pattern of FSH release, and do the local pituitary

regulatory factors fluctuate in a similar manner? What is the relative contribution of the peripheral inhibin and the local pituitary regulatory factors in modulating FSH secretion? A scenario can be envisioned in which the combined action of a number of factors (for example, inhibin, activin, follistatin and oestradiol) could lead to the appearance of FSH 'pulses' and an episodic pattern of FSH secretion (Fig. 5a).

If changes in local regulatory pituitary factors account for the non-GnRH-dependent episodic component of FSH secretion, changes in the interactions of these regulators and the consequent change in peripheral FSH must involve multiple intermediary steps (stimulus $\rightarrow$ changes in FSH regulatory proteins $\rightarrow$ FSH biosynthesis $\rightarrow$ FSH secretion). The time taken for these steps to be completed may result in the stimulus for a change in FSH release and the FSH release appearing unrelated or phase shifted in relation to the stimulus. For instance, suppressive effects of inhibin on FSH production and secretion in sheep are evident 4–6 h after inhibin administration (Wu *et al.*, 1987) and the decrease in plasma FSH is equivalent to the half-life clearance of FSH, indicating a shut-down of FSH release (McNeilly 1984). Although the effects on mRNA encoding FSH β are manifested sooner, other studies in male monkeys have shown a substantial lag between the time of administration of inhibin and peripheral FSH suppression (Ramaswamy *et al.*, 1998). This mode of regulation differs from the secretagogue (GnRH)-induced burst of LH and FSH release (stimulus secretion coupling).

Although changes in local pituitary factors may contribute to the appearance of an episodic pattern of FSH secretion, what other mechanisms can explain the non-GnRH associated pulses of FSH? What evidence is there in support of the existence of a second neural trigger (FSH-releasing factor; FSH-RF) (Fig. 5b) to account for the discordant pattern of episodic LH and FSH release?

Evidence supporting the existence of a separate FSH-releasing factor

Evidence for separate hypothalamic areas controlling LH and FSH secretion

Ablation (Lumpkin and McCann, 1984) and deafferentation (Lamperti and Hill, 1987) of the dorsal anterior hypothalamic area (DAHA) or electrochemical stimulation of hypothalamic regions apart from those that regulate LH secretion (Chappel and Barraclough, 1976) provide evidence for the existence of a specific site controlling FSH release. The strongest support for involvement of distinct neural regions for control of FSH pulsatility comes from studies of Lumpkin *et al.* (1989) demonstrating that radiofrequency lesions of the DAHA abolished FSH pulsatility without altering LH pulsatility. Selective suppression of FSH pulsatility is also observed after lesion of the caudal and mid-median eminence (Marubayashi *et al.*, 1999). These observations could be interpreted as indicating that an FSH-RF is produced in neurones with cell bodies in the DAHA with axons projecting into the caudal and mid-median eminence.

Fig. 5. Schematic representation of two mechanisms by which local pituitary factors or an FSH-releasing factor (FSH-RF) can account for the observed episodic pattern of FSH release. (a) Possible means by which changes in activin (a positive regulator of FSH) and inhibin–follistatin–oestradiol (negative regulators of FSH), in conjunction with GnRH, can account for GnRH-associated and non-GnRH-associated pulses of FSH. Although not represented in this figure, there is likely to be a time lag between changes in local tone and the manifestation of changes in FSH. In turn, these local factors appear to be regulated by GnRH. (b) Possible means by which GnRH, in conjunction with the putative FSH-RF, can account for GnRH-associated and non-GnRH-associated pulses of FSH.

Biochemical evidence for an FSH-releasing factor

Igarashi and McCann (1964) were the first to suggest the existence of a separate FSH-RF responsible for discordant FSH secretion. Initial separation of FSH-releasing activity from sheep hypothalami was achieved by gel filtration on a Sephadex G-25 column followed by carboxymethyl cellulose chromatography (Dhariwal *et al.*, 1965). In these studies, FSH-releasing activity emerged just before elution of the

classic GnRH. Differential separation methods were used by two groups to achieve clearer separation of FSH and LH-releasing activities from extracts of pig and sheep hypothalamus (Fuchs *et al.*, 1979; McCann *et al.*, 1983). Subsequent characterizations revealed that, while the bulk of the GnRH activity was localized to the anterior median eminence, the FSH-releasing activity of the posterior median eminence was much greater than could be accounted for by its GnRH content (Mizunuma *et al.*, 1983). A preparation enriched with biologically active FSH-RF free of GnRH was obtained subsequently by Lumpkin *et al.* (1987) after ion exchange chromatography of sheep hypothalamic extracts. Despite the recognized interest and importance of the topic and the mounting neuroanatomical, biochemical and physiological evidence supporting the existence of an FSH-RF in the hypothalamus, to date, a physiologically relevant FSH-RF has yet to be fully isolated and characterized.

Is a variant of GnRH the putative FSH-releasing factor?

Molecular advances in the identification of variant forms of GnRH open up the possibility that one form is the putative FSH-RF. The factor must be capable of selectively stimulating FSH over LH or at least be a more potent releaser of FSH than of LH to be categorized as an FSH-releasing factor. Nine forms of GnRH have been identified in lower vertebrates (Lin *et al.*, 1998) and a second form of GnRH has also been identified in higher mammals (Lescheid *et al.*, 1997; White *et al.*, 1998; Urbanski *et al.*, 1999). A putative receptor for this second form of GnRH has been identified in humans (Millar *et al.*, 1999). Although this second form of GnRH has been shown to release LH (Lescheid *et al.*, 1997), no information is yet available regarding its ability to release FSH.

Studies using rat hemi-pituitary cultures show that lamprey GnRH III releases FSH at much lower doses than those required for LH (Yu *et al.*, 1997). Testing *in vivo* showed a small selective increase (approximately 20%) in FSH over the saline controls, indicating that the lamprey GnRH-III is a homologue of mammalian FSH-RF, although more rigorous testing is required to establish whether this is the case. Such testing should include: (1) detailed dose-response studies *in vivo* in which the stress effects observed, for example, in the studies of Yu *et al.* (1997) can be minimized; (2) immunocytochemical localization of the counterpart of lamprey GnRH-III in the mammalian brain; (3) checking that the lesioning of areas where this variant GnRH localizes leads to selective elimination of FSH pulsatility (unless localization is restricted to sites, such as DAHA, implicated in the selective control of FSH); and, more importantly (4) documenting the expression of the gene encoding the counterpart of lamprey GnRH-III in the hypothalamus of higher vertebrates.

Conclusions

In summary, if the pivotal role FSH plays in controlling

Fig. 6. Schematic summary of the neuroendocrine control of FSH, showing the hypothalamic, ovarian and pituitary hormones involved in the control of FSH production and secretion. At the hypothalamus, GnRH is a known stimulator of FSH release. There is also evidence in support of the existence of a hypothalamic FSH-releasing factor (FSH-RF). At the pituitary, a local loop involving activin, inhibin and follistatin appears to be operational. At the ovary, oestradiol and inhibin are two major negative feedback regulators of FSH. Although the ovary produces both activin and follistatin, these hormones are not believed to play an endocrine role.

follicular development is considered, multiple regulatory and perhaps redundant mechanisms appear to be in place to ensure its production and release (Fig. 6). In addition to the established role of GnRH in stimulating FSH secretion, several other factors have been implicated as selective regulators of FSH release. Gonadal steroids and several FSH regulatory proteins including activins, inhibins and follistatins have major FSH regulatory roles, with oestradiol and inhibin both providing the primary inhibitory inputs. Studies indicate that activins, inhibins and follistatins are produced not only at the gonad but also at the pituitary and, therefore, have the potential to exert local modulatory effects on FSH secretion. Although neuroanatomical, biochemical and physiological evidence to date support the existence of a separate FSH-RF, this factor has yet to be isolated and purified.

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