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Follicle-stimulating isohormones: regulation and biological significance

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Follicle-stimulating hormone (FSH) is a key hormone in the regulation of follicular development. Although the existence of FSH heterogeneity is well established, the physiological significance of this pleomorphism remains unknown. Observed changes in circulating FSH heterogeneity during critical reproductive events such as puberty and reproductive cyclicity suggest that different combinations of FSH isoforms reach the target sites during different physiological states to influence a variety of biological end points such as cellular growth, development, steroidogenesis and protein synthesis. Considering that these FSH isoforms have different physicochemical properties and potential to bind not only their cognate receptors but also structurally related, non-FSH receptors with various affinities, the regulatory implications of FSH heterogeneity in modulating the various FSH-induced functions are enormous. However, assigning functional significance to FSH heterogeneity has been hampered because of (1) difficulties associated with procurement of highly purified, naturally occurring, circulating FSH isoforms; (2) absence of reference standards that contain the entire repertoire of FSH isoforms present in biological fluids; and (3) specificity issues inherent to the detection systems used. If particular FSH isoforms do possess selective biological functions, specific combinations of FSH isoforms could be generated to regulate fertility in farm animals and humans.

Introduction

Although the existence of protein heterogeneity is now uncontested, the intracellular mechanisms governing protein synthesis, post-translational modifications and post-secretion fates are poorly understood. Proteins emerging from the ribosome have a great variety of potential fates, depending on the presence or absence of critical amino acid sequences. These amino acid sequences constitute target signals that determine spatial configuration, folding and post-translational modifications (including the addition and trimming of carbohydrate units, stability, packaging in granules and secretion). Structural features (such as tertiary configuration, carbohydrate composition) determine the metabolic fate of the protein in the peripheral circulation. Although the structure of the oligosaccharide chains of glycoproteins is an important determinant of the circulating half-life, protein structure and exposed epitopes are important for target cell receptor binding and signal transduction. For these and other reasons, it appears that the resulting mix of heterogeneous hormonal isoforms exert effects at the target site that cannot be interpreted properly by conventional radioimmunoassays alone.

Existence, Origin and Functional Attributes of FSH Isoforms

Existence of FSH isoforms

Use of various separation techniques, such as chromatofocusing, isoelectric focusing and gel electrophoresis, has unequivocally established that FSH occurs in multiple forms both in the

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pituitary and circulation (reviewed in Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). The more discriminating the separation technique the more complex the distribution profiles of FSH isoforms. For instance, Burgon *et al.* (1993), using isoelectric focusing and high performance anion exchange chromatography, identified more than 20 human pituitary isoforms. A complete array of FSH isoforms ranging from basic to strongly acidic pI values is found both in the pituitary and circulation (reviewed in Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). In some physiological and pharmacological conditions, the distribution of circulating and pituitary FSH isoforms appears to be similar, while in others they differ.

Origin of FSH heterogeneity

There are several potential sites at which changes in FSH heterogeneity can originate. At the pituitary, the array of FSH isoforms that exist during different physiological states may represent merely FSH at different stages in the biosynthetic pathway. Alternatively, changes in FSH heterogeneity may be the outcome of alterations in post-translational processing and a function of glycosylation changes. In this context, it is of interest that the activities of glycosyltransferases and sialotransferases, enzymes involved in sulphation and sialylation of luteinizing hormone (LH) and FSH, vary with the physiological state in the pituitary (Dharmesh and Baenziger, 1993; Damián-Matsumura *et al.*, 1998). This may contribute to potential differences in glycosylation of pituitary gonadotrophins.

At the circulatory level changes in FSH heterogeneity may originate from regulated preferential secretion, peripheral modification, or differences in metabolic clearance. *In vitro* studies (reviewed in Chappel *et al.*, 1983; Ulloa Aguirre *et al.*, 1995) and recent *in vivo* studies in sheep (Lee *et al.*, 1998) suggest that the pituitary has the ability to secrete different combinations of FSH isoforms depending on the physiological status of the animal.

Functional attributes of FSH isoforms

The nature of the oligosaccharide side chains varies considerably and may be manifested as changes in carbohydrate content, length and branching of the side chain and the associated charge. These structural differences appear to dictate the receptor binding ability, *in vitro* and *in vivo* biological activities, and circulatory half-lives of the FSH isoforms. Most of the earlier work that used biological to immunological ratios to estimate FSH biopotencies concluded that *in vitro* biological activity of the FSH isoform is positively correlated with the pI value of the isohormone; the FSH isoforms with higher pI values have higher *in vitro* biological activities than those with lower pI values (reviewed in Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). An exception to this rule is that the basic FSH isoforms that elute in the void volume of the chromatofocusing column (pH gradient 7.4–4.0) appear to act as antagonists of FSH (Dahl *et al.*, 1988; Timossi *et al.*, 1998).

The assumption in the use of B:I ratios for estimating FSH biopotency has been that the immunopotencies are similar between different FSH isoforms. More recent studies that used highly purified FSH isoforms of defined mass (not adjusted for carbohydrate content and oxidative losses) have raised questions about this assumption and shown the contrary, namely that FSH immunopotencies of different FSH isoforms vary (Burgon *et al.*, 1993; Stanton *et al.*, 1996). It then follows that earlier biopotency estimates that used immunoassays to determine gonadotrophin protein mass may be inaccurate. On the basis of the high correlation between immuno-, radio-receptor and *in vitro* bioactivity of 15 purified human FSH isoforms (> 90% purity) in the pI value range of 3.63–5.13, Burgon *et al.* (1993) concluded that: (1) current immunoassays are measuring mass in combination with some measure of bioactivity and (2) an immunoassay directed to the 'invariant region of the molecule' not affected by glycosylation differences is required to assess the true bioactivity of the FSH isoform. Interestingly, in spite of the high correlation between the

immuno-, radio-receptor and *in vitro* bioactivities of individual isoforms, these investigators also noted significant differences in ratios of activities between *in vitro* bioassays and other methods (Burgon *et al.*, 1993). For example, the *in vitro* bio- to radio-receptor assay ratio of the human FSH isoform with a pI value of 4.23 was four times greater than those with pI values of 3.63, 3.88, 4.07, 4.85, and 5.13. Isoforms with higher pI values, such as those reported to have antagonistic properties (Dahl *et al.*, 1988; Timossi *et al.*, 1998) or the less acidic FSH isoforms (pI > 5.4) seen during puberty (Padmanabhan *et al.*, 1992), have not been evaluated. Differences in assay ratios from the carefully characterized study of Burgon *et al.* (1993) and the 5–8-fold differences in immuno-, radio-receptor and *in vitro* bioactivities of the various human FSH isoforms indicate that structural heterogeneity of FSH does contribute to functional differences.

In terms of the circulatory half-life, many studies using chemically or enzymatically modified FSH have shown that the less acidic FSH isoforms have shorter half-lives than the more acidic FSH isoforms (reviewed in Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). Differences in half-life in these instances appear to relate to the degree of sialylation (Morell *et al.*, 1971). Although most studies follow this general rule, others have failed to observe such relationships (Robertson *et al.*, 1991). Assuming that the FSH isohormones with shorter circulatory half-lives have higher biological activity *in vitro*, the issue is to determine whether measures of *in vitro* biological activity are meaningful in interpreting consequences *in vivo*. Studies by Stanton *et al.* (1996) show a 16-fold range in *in vivo* activities between various LH isoforms. These changes parallel estimates of bioactivity *in vitro*. This finding suggests that differences in bioactivity *in vitro* and not the large differences in circulatory half-life are the key determinants of bioactivity *in vivo* as assessed in short-term assays (long-term consequences were not assessed).

The effector mechanisms by which FSH elicits its target cell action appear also to be conducive for rapid functional interactions between FSH and its receptor. For example, minutes after addition, FSH elicits increases in intracellular calcium (Flores *et al.*, 1990). Furthermore, effective activation of adenylate cyclase appears possible under conditions of intermittent receptor activation (Spiegel *et al.*, 1992). In this context it is of interest that FSH is secreted both in a basal and pulsatile mode (Padmanabhan *et al.*, 1997a). Some evidence also indicates that what is secreted in pulses is of different molecular nature than that secreted in the basal mode (reviewed in Ulloa-Aguirre *et al.*, 1995). It still remains to be addressed whether the pulsatile inputs are perceived by the target site as such, and if so, do they respond differently to this intermittent activation as opposed to FSH secreted in the basal mode.

Although many issues still remain to be addressed, it is clear that a combination of circulating gonadotrophin isoforms reaches target tissues and influences a variety of biological end points such as cellular growth, development, steroidogenesis and synthesis of proteins. FSH isoforms possessing prolonged circulation times may have the potential to provide a long-acting stimulus for the progression of maturational events. In contrast, isoforms with shorter half-lives that are secreted intermittently may provide an acute yet potent stimulus (Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991). Relative proportions of the various gonadotrophin isoforms within the circulation, therefore, have the potential to exert qualitatively different effects on target tissues.

Methodological Considerations in Assessing FSH Heterogeneity

Utility of B:I ratios

Initial studies of FSH heterogeneity relied heavily upon comparative bioactivity (B) and immunoreactivity (I) estimates. In these instances changes in B:I ratios were taken to imply that there was a change in the constituent mix of FSH isoforms. More recent studies have questioned this concept (Jaakkola *et al.*, 1990, Simoni *et al.*, 1994). Conclusions on hormonal heterogeneity derived from studies of B:I ratios of FSH must be drawn with caution and may suffer from two major drawbacks. First, it needs to be proven that the differences in measured bioactive FSH and immunoreactive FSH are not simply the result of differences in the effects of interfering substances

on the measurement system but result from an ability to discriminate among isoforms. For example, RIAs using different combinations of standards and antisera (with different epitopic recognition) can differentially recognize gonadotrophin mixtures (Simoni *et al.*, 1994). Bioassays suffer in that substances present in the test material can interfere (Simoni *et al.*, 1991) or modulate the effectiveness of FSH (reviewed in Beitins and Padmanabhan, 1991; Chappel, 1995) leading to erroneous estimates.

Second, the FSH standards used in various immuno- and bioassays add another source of error when estimating changes in FSH B:I ratios. The distribution patterns of FSH isoforms of pituitary and urinary FSH standards appear to have sparser representation of less acidic FSH isoforms than those found in circulation and unpurified pituitary extracts (Simoni *et al.*, 1993a, 1993b). This finding suggests that the standards that are currently in use in the various assays do not have the full array of FSH isoforms that are present in biological fluids. Although it is ideal to have a reference preparation that contains the repertoire of FSH isoforms present in the biological fluid being measured, this does not appear practical in view of the high variability associated with its composition during different physiological states. At the very minimum, documentation of changes in FSH heterogeneity using FSH B:I ratios requires careful characterization of the assay systems and use of the same reference preparations. Unfortunately, many of the earlier studies reporting changes in FSH B:I ratios used different FSH standards to estimate the immuno- and biopotencies. In fact, the first study that challenged the utility of B:I ratios itself used two different standards in their immuno- and bioassays (Jakkola *et al.*, 1990). The specificity of the monoclonal antibodies also adds an additional problem in that they fail to recognize all variants (Pettersson and Soderholm, 1991).

In terms of bioassays, the biopotency estimate of the FSH isoform may also vary with the biological end point or assay system chosen. When cAMP is used as the endpoint in the Sertoli cell bioassay, deglycosylated ovine FSH appears to be inactive (Fig. 1) (Padmanabhan, 1995). On the contrary, when oestradiol is chosen as the end point, deglycosylated FSH stimulates oestradiol production. Species specificity of the bioassay is also a consideration. For instance, Ding *et al.* (1991) have shown that the bioactive LH estimates and the B:I ratios vary markedly depending on whether the mouse Leydig cell, rat interstitial cell or human granulosa cell bioassay is used to measure LH bioactivity. Bioassays of FSH that use cells transfected with recombinant FSH receptors (Gudermann *et al.*, 1994; Christine-Maitre *et al.*, 1996) and cAMP as the end point also pose a problem because they do not measure a biological end point and ignore involvement of other second messenger systems involved in FSH signal transduction.

Taking all aforementioned caveats into consideration, it appears that differences in FSH B:I ratios reported by different investigators during similar physiological situations are a direct function of the assay, the standard and end point used. For instance, FSH B:I ratios are constant throughout the cycle (Jia *et al.*, 1986), highest during the preovulatory period (Padmanabhan *et al.*, 1988a; Wide and Bakos, 1993; Zambrano *et al.*, 1995), or maximal in the luteal phase (Christine-Maitre *et al.*, 1996) of the human menstrual cycle. Therefore, when comparing biological potency estimates, it is important to consider the species, the cellular types, as well as the relative endpoints of the bioassay used by the various investigators. Assuming that assay systems are controlled carefully, changes in B:I ratio at best will point to possible changes in heterogeneity but will require confirmation with other chromatographic approaches.

Chromatographic separation

Several fractionation approaches have been used effectively to characterize changes in FSH heterogeneity and have been reviewed extensively (Ulloa-Aguirre *et al.*, 1995). Although a broader classification of FSH isoforms can be achieved by gel permeation chromatography, higher resolution has been achieved with techniques that separate on the basis of charge. Some charge-based techniques that have been used extensively are isoelectric focusing, chromatofocusing and analytical zone electrophoresis. These approaches while providing a general assessment of changes, do not separate isoforms to purity which requires multiple chromatographic steps (Burgon *et al.*, 1993). An inherent caveat in these approaches is that estimates of FSH isoform distribution after fractionation are based on immunoassay systems that have their inherent problems. Recent studies of Simoni *et al.*

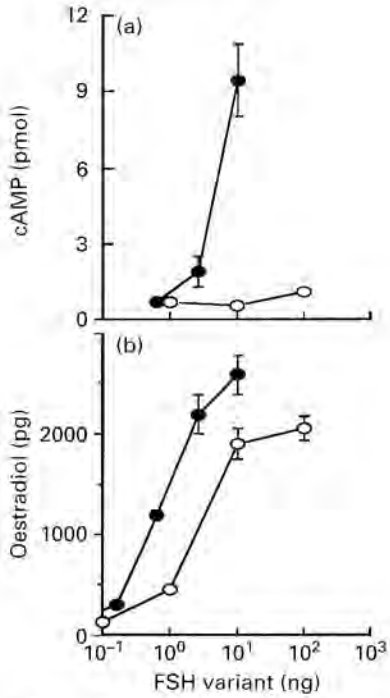

Fig. 1. (a) cAMP, and (b) oestradiol responses of immature rat Sertoli cells to increasing concentrations of native (oFSH) (●) or deglycosylated oFSH (DG-oFSH) (○). Sertoli cells from 7–10-day-old rats were cultured using previously validated methods (Padmanabhan *et al.*, 1987). Cell monolayers were exposed to increasing concentrations of oFSH or DG-oFSH for 24 h. Thirty minutes after addition of FSH, a 200 µl aliquot of medium was removed from each well for cAMP determinations. Medium concentrations of oestradiol (24 h incubates) and cAMP (30 min incubates) were measured using previously validated assays. Note the differences in cAMP and oestradiol responses to the native and deglycosylated FSH (modified from Padmanabhan *et al.*, 1993).

(1994) have shown that the distribution profiles of FSH after chromatofocusing vary considerably between immunoassays because each assay recognizes individual isoforms of FSH differently.

Regulation of FSH Heterogeneity

A central theme emerging from investigations of FSH heterogeneity is that the endocrine status regulates the proportion of various FSH isoforms and that these isoforms show distinct actions.

Although the factors affecting the final distribution of gonadotrophin isoforms within the circulation are multifaceted and complex, it is clear that endocrine changes regulate the proportions of FSH isoforms both within the pituitary and in the peripheral circulation.

Pituitary FSH heterogeneity

Endocrine regulation of pituitary FSH microheterogeneity has been studied widely in numerous species. Several reviews have addressed the regulation of pituitary heterogeneity in great detail (reviewed in Chappel *et al.*, 1983; Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). Qualitative differences in pituitary FSH content have been correlated with age, sex and stage of the oestrous cycle in several species by using various techniques (Blum and Gupta, 1980; Chappel *et al.*, 1983; Ulloa-Aguirre *et al.*, 1995). In general, pituitary FSH is less acidic in female than male pituitaries and young than old animals. Changes in pituitary FSH heterogeneity (predominance of less acidic FSH isoforms) have also been found during onset of puberty in rats (Chappel *et al.*, 1983), but not in heifers (Stumpf *et al.*, 1992).

Circulating FSH heterogeneity

Regulation of circulating FSH heterogeneity has not received such intense investigation as pituitary heterogeneity, in part due to limitations imposed by relatively low concentrations of circulating FSH. Development of sensitive, *in vitro* FSH bioassays which use oestradiol as the end point (granulosa cell bioassay: Jia *et al.*, 1986; Sertoli cell bioassay: Padmanabhan *et al.*, 1987) have allowed characterization of changes in circulating bioactive FSH during different physiological states (reviewed in Beitins and Padmanabhan, 1991). Because of the caveats discussed above in using B:I ratios as an index of changes in FSH heterogeneity, in this report, only those studies that used chromatographic approaches either alone or in conjunction with bioactivity measures to assess changes in FSH heterogeneity will be considered. In general, increases in oestradiol and GnRH in gonad-intact models lead to increases in less acidic FSH isoforms and corresponding increases in bioactive FSH in the circulation (Fig. 2) (reviewed in Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). Administration of GnRH to prepubertal boys (Phillips and Wide, 1994), men (Simoni *et al.*, 1996), and women (Zambrano *et al.*, 1995) increases the release of less acidic FSH isoforms. Treatment of women with GnRH antagonist, on the other hand, leads to an increase in the circulation of a highly basic FSH isoform (Dahl *et al.*, 1988). This basic FSH isoform was shown to antagonize the action of FSH.

Few studies have also addressed the role of oestradiol in modulating circulating FSH heterogeneity. Studies in humans documenting changes in FSH heterogeneity (shift to a less acidic side) following treatment of postmenopausal women (Wide and Naessén, 1994) or a Turner's girl (Padmanabhan *et al.*, 1988a) with oestrogens provide corroborative evidence in support of a role for oestradiol in modulating circulating FSH heterogeneity. In these instances, since oestrogens can induce their effects via mediation of hypothalamic GnRH secretion, it is difficult to separate the direct effects of oestradiol from mediation via alterations in GnRH input. Studies using ovariectomized, nutritionally growth-retarded (hypogonadotrophic) sheep have shown that pulsatile GnRH administration alone, in the absence of oestradiol, increases LH but does not alter the distribution profile of FSH or bioactive FSH secretion (Fig. 3) (Hassing *et al.*, 1993). In contrast, oestradiol administration to nutritionally growth-retarded lambs leads to an increase in less acidic FSH isoforms (Padmanabhan *et al.*, 1997b) (Fig. 4). Therefore, these findings support a role for oestradiol in mediating FSH heterogeneity.

Progesterone and androgens appear to have an opposite effect to that of oestradiol since they appear to increase the presence of more acidic FSH isoforms (Fig. 2) (reviewed in Ulloa-Aguirre *et al.*, 1995). In the presence of high progesterone, oestradiol fails to increase the presence of less acidic FSH isoforms in the circulation (Wide *et al.*, 1996a). Furthermore, the predominant circulating form of FSH is acidic during the luteal phase of the human menstrual cycle (Padmanabhan *et al.*, 1988a) and

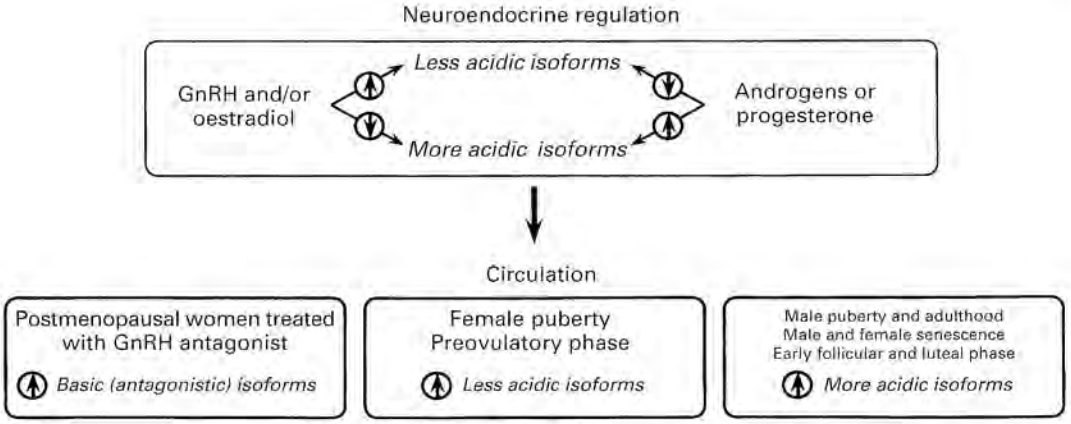

Fig. 2. Schematic diagram summarizing the neuroendocrine regulation of FSH heterogeneity and the nature of circulating FSH isoforms during different physiological states (reviewed in Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995). GnRH (particularly in ovary-intact models) and oestradiol appear to increase the proportion of less acidic FSH isoforms and decrease the presence of more acidic FSH isoforms. In contrast, androgens and progesterone appear to increase the concentration of more acidic FSH isoforms. The distribution of FSH isoforms also varies with the physiological state. For example, during the onset of puberty in females and the preovulatory phase greater proportions of less acidic FSH isoforms circulate. In contrast, circulating FSH in males, during female senescence, and the early follicular and luteal phases of the reproductive cycle appear predominantly acidic in nature. Interestingly, treatment of post-menopausal women with a GnRH antagonist increases the release of basic FSH isoforms with antagonistic properties (Dahl *et al.*, 1988).

the prepartum period in cattle (Crowe *et al.*, 1998), when concentrations of progesterone and oestradiol are both high.

In all these studies it is difficult to ascertain whether an increase in less acidic FSH isoforms such as those induced by GnRH or oestradiol is due to selective secretion or metabolic alteration. Studies of Harsch *et al.* (1993) suggest that metabolic deglycosylation can occur in circulation. More recent studies characterizing FSH distribution near the site of secretion show that oestradiol selectively increases the secretion of less acidic FSH isoforms (Lee *et al.*, 1998). Evidence is also accumulating to show that oestradiol alters the activity of pituitary glycosyl- and sialotransferases (Dharmesh and Baenziger, 1993; Damian-Matsumura *et al.*, 1998), thereby contributing to glycosylation differences. Overall changes in circulating FSH heterogeneity appear to be the sum effect of secretory changes, metabolic alterations and metabolic clearance.

Biological Significance

When assessing biological significance, it is not sufficient only to show FSH heterogeneity is present and is regulated. It is essential to assess whether such changes are biologically meaningful and to determine whether the naturally occurring FSH isoforms are different functionally. In general, increased release of less acidic FSH isoforms occurs at the onset of puberty and the preovulatory period (Fig. 2). In contrast, more acidic FSH isoforms predominate in males, during senescence in both males and females, and during the early follicular and luteal phases of the oestrous or menstrual cycles (Fig. 2) (reviewed in Beitins and Padmanabhan, 1991; Ulloa-Aguirre *et al.*, 1995).

Changes in FSH heterogeneity may be important in the pubertal process

Experimental induction of puberty in female lambs increases the release of circulating bioactive FSH in pubertal lambs as compared with prepubertal lambs; this increase is not evident when

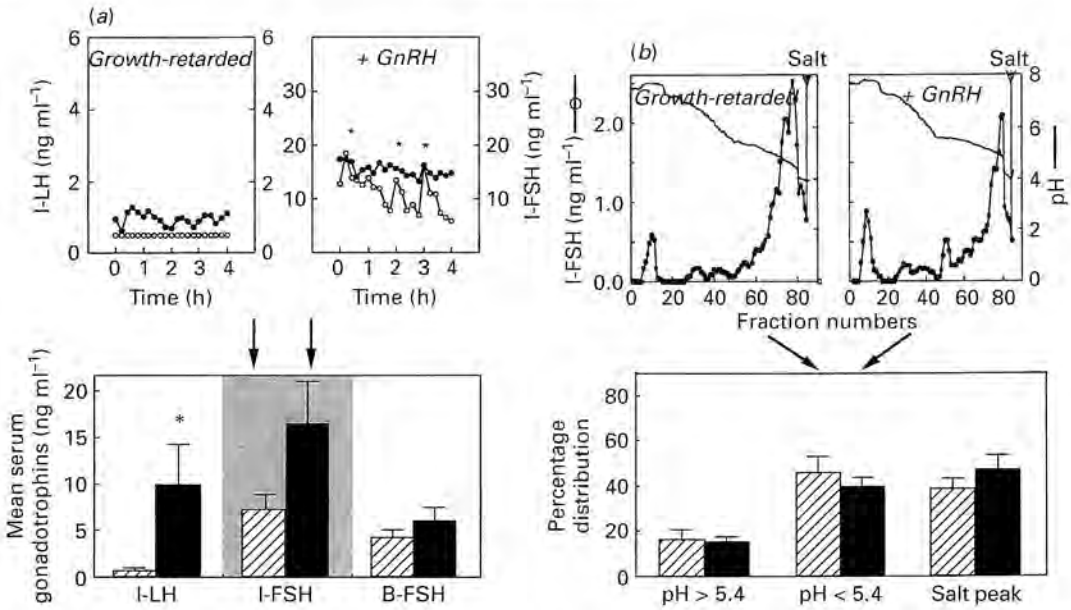

Fig. 3. Changes in circulating concentrations of gonadotrophins and FSH heterogeneity after pulsatile administration of GnRH to ovariectomized nutritionally growth-retarded (hypogonadotrophic lamb) (Hassing *et al.*, 1993). (a) Circulating patterns of immunoreactive (I) FSH in a growth-retarded and GnRH-treated lamb (top) and mean concentrations of I-FSH and bioactive FSH (B-FSH) measured by the Sertoli cell aromatase bioassay (bottom). Circulating patterns and mean concentrations of I-LH are provided for comparison. (b) Distribution pattern of immunoreactive FSH (closed circles) after separation of serum from a representative growth-retarded (left) and GnRH-treated (right) lamb by chromatofocusing (top). Percentage distribution of circulating FSH isoforms that eluted at pH above 5.4, below pH 5.4 and the components bound at the lower limiting pH are shown in the bottom. Values represent mean ± SEM ($n = 4$ for each group).

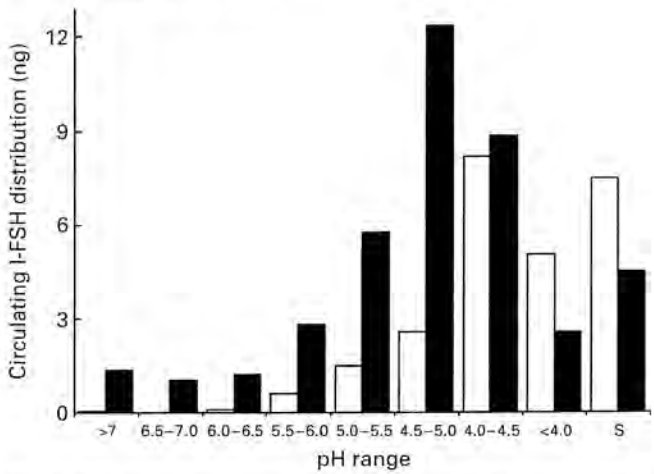

Fig. 4. Distribution pattern of immunoreactive FSH after separation of serum from a representative ovariectomized hypogonadotrophic (□) or oestradiol-treated (■) lamb by chromatofocusing. Ovariectomized hypogonadotrophic lambs were treated with follicular phase concentrations of oestradiol (via implants for 18 h). Note the shift to the less acidic side of FSH distribution in the oestradiol-treated lamb (Padmanabhan *et al.*, 1997b).

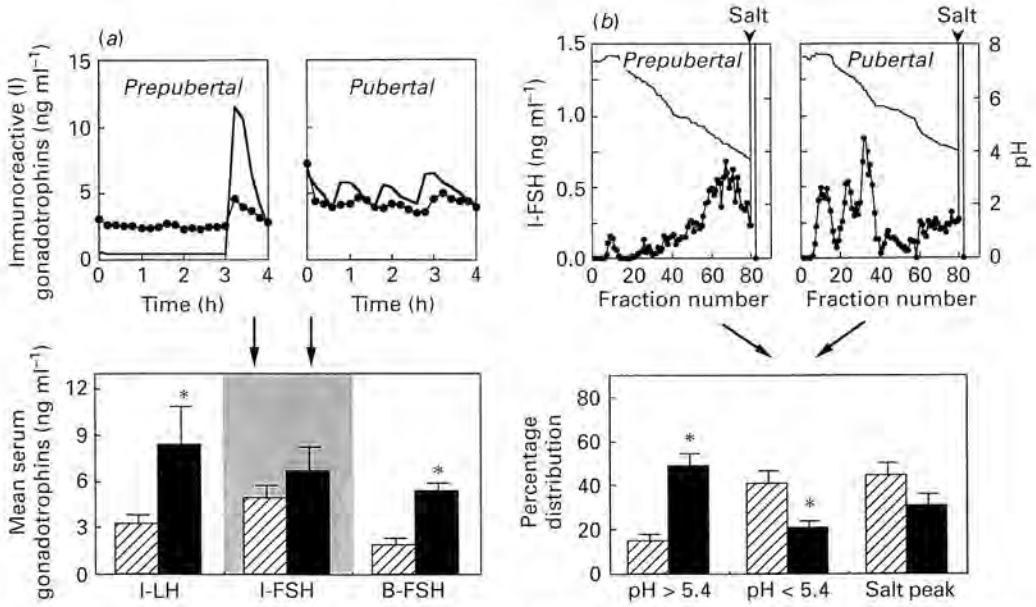

Fig. 5. Changes in circulating concentrations of gonadotrophins and FSH heterogeneity during experimental induction of puberty in female lambs (Padmanabhan *et al.*, 1992). (a) Shown are circulating patterns of immunoreactive (I) FSH in a prepubertal and pubertal lamb (top) and mean concentrations of I-FSH and bioactive FSH (B-FSH) measured by the Sertoli cell aromatase bioassay (SAB) and granulosal cell aromatase bioassay (GAB) in the prepubertal and pubertal lambs (bottom). Circulating patterns and mean concentrations of I-LH are also provided for comparison. Note the selective increase in B-FSH as measured by the SAB and GAB assays at the onset of puberty. (b) Distribution pattern of I-FSH (closed circles) after separation of serum from a representative prepubertal (left) and pubertal (right) lamb by chromatofocusing (top). Note the elution of greater amounts of immunoreactive FSH isoforms in the less acidic region in the pubertal lamb. Percentage distribution of circulating FSH isoforms that eluted at pH above 5.4, below pH 5.4 and the components bound at the lower limiting pH are shown in the bottom. Values represent mean ± SEM (n = 4 for each group).

measured by a radioimmunoassay (Fig. 5) (Padmanabhan *et al.*, 1992). The increased release of bioactive FSH in pubertal lambs is evident whether measured by the Sertoli or the granulosa cell bioassays. However, quantitative differences in bioactive FSH estimates are evident depending on the bioassay used (reviewed in Ulloa-Aguirre *et al.*, 1995). The increased release of B-FSH in pubertal lambs also is accompanied by a change in the distribution pattern of circulating FSH isoforms (Fig. 5) with increased release of less acidic (pI > 5.4) serum FSH isoforms. Considering that pubertal onset is associated with increased gonadal activity, the less acidic FSH isoforms in conjunction with increased secretion of bioactive FSH indicate that the less acidic isoforms may be biologically meaningful and have the potential to provide a potent and acute signal to the ovary. Such changes may be the consequence of increased oestradiol secretion from the ovary.

Changes in FSH heterogeneity may be important in the ovulatory process

Detailed chromatographic studies characterizing the changes in circulating FSH heterogeneity during the ovulatory cycles have been addressed in humans (Padmanabhan *et al.*, 1988a; Wide and Bakos, 1993; Zambrano *et al.*, 1995) and cattle (Cooke *et al.*, 1997). These studies reveal that increases in less acidic circulating FSH isoforms occur during the preovulatory period. Studies using concanavalin chromatography show that the complexity of the oligosaccharide chains are also altered during different phases of the human menstrual cycle and that less complex FSH isoforms

are found during mid-cycle than in early follicular or late luteal periods (Anobile *et al.*, 1998). The marked shift in FSH distribution profile favouring less acidic isoforms of FSH coincides with the timing of preovulatory follicular development.

FSH isoforms may differ in their functional attributes

Before attributing physiological significance to heterogeneity, it is essential to determine whether the various isoforms differ in their functionalities. To be biologically meaningful, changes in FSH heterogeneity need to be of sufficient magnitude to alter the net potency of the hormone or have various functions. Estimations of the biopotencies of FSH isoforms on the basis of FSH mass predict a 5–8-fold difference in radio-receptor and biopotency of purified human pituitary-derived FSH isoforms as well as a fourfold discrepancy between radio-receptor and *in vitro* biopotencies (Burgon *et al.*, 1993). Considering that subtle increases in immunoreactive FSH can induce ovarian responses (Ben-Rafael *et al.*, 1995), potency differences such as those reported by Burgon *et al.* (1993) and the magnitude of changes such as those seen during mid-cycle (Padmanabhan *et al.*, 1988a) and puberty (Padmanabhan *et al.*, 1992) have the potential to have meaningful biological consequences. In this context it is of interest that pulsatile administration of GnRH leads to fast clearing FSH signals in patients with idiopathic hypogonadotropic–hypogonadism (Padmanabhan *et al.*, 1988b) and a less negatively charged (less acidic) form of FSH in children with pubertal disorders (Wide *et al.*, 1996b). Similarly treatment with a GnRH antagonist leads to production of a basic form of FSH that is capable of antagonizing FSH action (Dahl *et al.*, 1988).

Less acidic (pI 5.0–5.6) isoforms of human recombinant FSH have been shown to be more potent than those in the mid- (pI 3.6–4.6) and acidic (pI 4.5–5.0) ranges in inducing mouse follicular development *in vitro* (Vitt *et al.*, 1997). In addition to inducing follicles of large final size, the less acidic FSH isoforms, even at the lowest concentration tested (25 mIU ml^{-1}), induced antral formation in 70% of follicles and oestrogen production by day 2 of exposure to FSH. A similar degree of follicle development was achieved only with 100 mIU ml^{-1} of the mid- pI value and 500 mIU ml^{-1} of acidic FSH isoforms. Furthermore, oestradiol secretion was evident only after 4 days of culture even with the 500 mIU acidic FSH ml^{-1}. These studies indicate that the physicochemical nature of FSH isoforms may lead to quantitative and qualitative differences in ovarian function.

An exciting possibility is that the different FSH isoforms encode different functions. On the basis of the multitude of functions FSH mediates at the gonads, involvement of different second messenger systems and potential for receptor cross-talk (Fig. 6), the signal transduction pathway of FSH isoforms may differ and culminate in altered responses. There is precedence with other glycoprotein hormones for such altered signal transduction cascades. FSH and thyroid-stimulating hormone (TSH) have been shown to bind each others receptors (Dobozy *et al.*, 1985). Interaction of TSH with human FSH receptor is a possible mechanism by which some children with juvenile hypothyroidism exhibit unexplained precocious puberty (Anasti *et al.*, 1995). Asialo human chorionic gonadotrophins (hCGs) have been shown to have higher TSH-like activity in human thyroid follicles (Yamazaki *et al.*, 1995). In this context, it is of interest that there is a positive correlation between serum free thyroxine and asialo hCG in patients with gestational thyrotoxicosis (Tsuruta *et al.*, 1995). Similarly, different isoforms of TSH have been shown to have different biological activities: the basic isoforms promoting iodide and thymidine uptake and acidic isoforms increasing intracellular cAMP (Pickles *et al.*, 1992). Certain LH isoform(s) have also been shown to possess renotrophic activity but weak steroidogenic potential (Nomura *et al.*, 1988). Studies with deglycosylated and native mixes of ovine FSH also show isoform specific cAMP and oestradiol responses (Fig. 1) (Padmanabhan, 1995). These observations provide support for the possibility that endocrine-induced changes in FSH heterogeneity can have functional consequences.

Conclusion

Our understanding of mechanisms controlling the pubertal process and ovulatory cyclicity may be flawed, in as much as the basis for such concepts is derived mainly from immunological

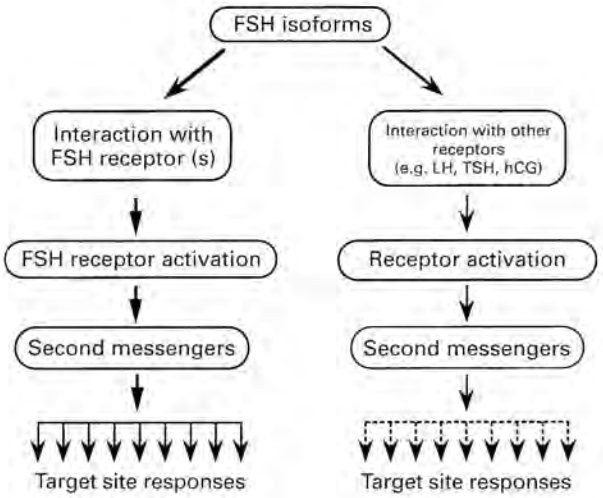

Fig. 6. Schematic diagram showing the potential sites at which the effector mechanisms may vary for the different FSH isoforms leading ultimately to differential target site responses. Different FSH isoforms may show differential affinity to a given FSH receptor, or selectively bind variant FSH receptor population or even structurally unrelated receptors (receptor cross-talk). Changes in receptor binding in turn can lead to changes in signalling cascades involving different second messenger systems and culminate in differential target cell responses.

characterizations of circulating patterns of gonadotrophic hormones that may not recognize the entire repertoire of gonadotrophin variants present in circulation. Mounting evidence indicates that, through an interaction with the hypothalamus and the gonad, the anterior pituitary gland can secrete different types of FSH that vary in biological potency and circulatory half-life. This opens up very interesting questions such as whether target cells differentially respond to the pattern of the imposed FSH signals, much as the T cell is known to differentially respond to small changes in ligand (Marx, 1995). In addition, the molecular modifications responsible for these observed changes in the distribution of FSH isoforms have the potential to lead to changes in affinity for classical FSH receptors, cross-reactivity with non-FSH receptors as well as with any other FSH receptor forms that might have been induced by the same endocrine conditions that led to the changes in FSH. From these perspectives, changes in FSH heterogeneity have the potential to provide an exquisitely fine-tuned mechanism to control gonadal function.

Therefore, it is a matter of great importance: (1) to determine details of the linkage between changes in isoform distribution and modifications in the biological attributes of the FSH signal delivered to the gonad during different physiological and pathological states; such efforts need to use well characterized assay systems and reference preparations that are reflective of the repertoire of FSH isoforms present in biological fluids, and (2) to reveal whether the various isoforms differentially act to initiate distinct functions in target cells. Only when the structure and importance of the various naturally occurring FSH isoforms are determined will it be possible for us to obtain a complete understanding of the mechanisms regulating reproductive processes. If heterogeneity proves to be biologically important, it should be possible to design FSH isoforms for desired functions and to use them too regulate fertility.

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