The role of pituitary gonadotrophin-releasing hormone receptors in the physiological regulation of gonadotrophin secretion

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Physiological regulation of pituitary gonadotrophin secretion

The decapetide gonadotrophin-releasing hormone (GnRH) is now regarded as the sole hypothalamic product governing the release of the pituitary gonadotrophic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). After synthesis in the perikarya of hypothalamic neurons, GnRH is transported along axons to be stored in the nerve terminals of the median eminence region of the hypothalamus. GnRH release into hypophyseal-portal capillaries is effected by neurotransmitters of the central catecholaminergic system, originating in many brain areas [1, 2].

In males, the serum level of gonadotrophic hormones is under classical negative feedback control from gonadal steroids. However, in females, oestrogen feedback regulation of LH is both negative and positive, this latter being unique to the gonadotrophins and with no male counterpart. Rising serum oestrogen concentrations from the maturing ovarian follicle sensitize the gonadotroph to GnRH stimulation, with resultant augmentation of LH release during mid-cycle. The oestrogen-induced LH discharge is responsible for follicle rupture and ovulation, without which cyclical ovarian activity ceases and infertility ensues.

Studies in vivo to define the precise site(s) of positive oestrogen feedback have largely been performed in intact subjects or animal models. These are difficult to interpret since oestrogen could act primarily either to modify hypothalamic GnRH secretion, or directly upon the pituitary gonadotroph to enhance its responsiveness to endogenous GnRH.

There is considerable evidence in vivo and in vitro for both inhibitory and stimulatory influences of gonadal steroids at the level of the pituitary gland. Much less information is available concerning sex steroid regulation of hypothalamic GnRH secretion in different physiological circumstances, since the peptide cannot be measured reliably in systemic serum samples. However, from measurements of GnRH in blood collected from the pituitary stalk of animals, a technically difficult approach, evidence is emerging that tonic negative feedback of gonadotrophin secretion could operate at the level of the hypothalamus [3, 4]. Cyclical oestrogen augmentation of LH release might also be mediated, at least in part, by its stimulation of endogenous GnRH release, at least in some species [5].

However, in primates the major site for oestrogen positive feedback is probably the pituitary itself. When endogenous GnRH secretion is eliminated by destructive lesions of the hypothalamic median eminance, gonadotrophin release and cyclical ovarian activity promptly ceases. Cyclical ovarian steroid secretion and pre-ovulatory serum LH surges, identical with those of a spontaneous cycle, as well as ovulation, can be restored by a regimen of unvarying exogenous GnRH administration [6, 7]. Thus, the primate menstrual cycle can be generated by ovarian steroids acting solely upon the anterior pituitary with hypothalamic GnRH secretion serving an essential permissive, rather than primary regulatory, role. Convincing though these elegant studies appear, they do not exclude oestrogen amplification of endogenous GnRH secretion in the intact primate.

Serum levels of gonadotrophins in adult animals fluctuate in a pulsatile fashion with a
periodicity of approximately 1–2 h. This probably reflects episodic release of hypothalamic GnRH. Therefore, analysis of LH pulse frequency may provide an indirect estimate of hypothalamic function. On this basis, luteal-phase progesterone secretion reduces the frequency of endogenous GnRH secretion [8, 9]. On the other hand, constant levels of oestrogens do not alter the frequency of gonadotrophin pulses but reduce their magnitude, implying either reduction in the frequency of gonadotrophin secretion at the pituitary level. It is not known whether the changing oestradiol levels characteristic of the follicular phase of the menstrual cycle can provoke changes in the frequency of GnRH discharges. An essential role for the central nervous system, presumably through the final pathway of endogenous GnRH secretion, is evident from a number of clinical situations: 'psychogenic' amenorrhoea/oligo-menorrhoea including anorexia nervosa (AN); weight-loss-related amenorrhoea (without the psychiatric features of AN); the onset of puberty, when the early hallmark is nocturnal pulsatile gonadotrophin secretion.

Much research effort has been applied to further our knowledge of the hormonal regulation of the menstrual cycle, with the implication that a greater understanding might lead to improved and safer methods for its interruption. In the last few years the physiological significance of pituitary exposure to intermittent, rather than continuous, stimulation by GnRH has been appreciated [6]. It has now been clearly shown that continuous administration of GnRH [10], or even intermittent exposure to high doses, especially of the long-acting agonist analogues [11], causes a progressive reduction in pituitary gonadotrophin output. This pituitary refractoriness, or desensitization, applies to many species, and also to cultured pituitary cells in vitro [12]. The therapeutic implications of these observations are discussed later in this review.

**Mechanism of GnRH action**

The subcellular mechanism(s) through which the feedback influences of gonadal steroid hormones might operate upon pituitary responsiveness to GnRH are unclear. Despite much research over the last 10 years, consensus has only been reached on the first and last steps in the process of LH release following GnRH stimulation.

In common with many other small peptide and glycoprotein hormones, GnRH initiates its effects following interaction with specific receptors in the gonadotroph surface membrane [13].

Following hormone receptor binding a series of plasma membrane-related events occur which ultimately lead to LH secretion, and probably also synthesis (Table 1). No single intracellular biochemical marker of GnRH–receptor interaction has been identified as a 'second messenger' which increases before release of LH. However, there is agreement that an increase in intracellular Ca²⁺ concentration is essential for GnRH-mediated LH release [14]. Although the precise intracellular site of Ca²⁺ action requires clarification, participation of calmodulin in Ca²⁺-induced enzyme activation and exocytosis of secretory granules has been implicated [15]. Cyclic nucleotides [adenosine cyclic 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP)] are elevated following GnRH action, though this occurs subsequent to LH release and therefore does not meet the criteria for a second messenger [16–18]. There are increases in arachidonic acid metabolism and membrane phospholipid turnover, which accompany GnRH-stimulated LH release, though their precise relationship to the release process is unclear [19]. The gonadotroph cytoskeleton seems important, since intact microfilament function appears necessary for maximal LH release [20]. Recently, it has been shown that the LH release process is independent of cellular internalization of GnRH–receptor complexes by the gonadotroph [21]. Although none of these many biochemical subcellular events, with the exception of Ca²⁺ mobilization, is directly linked to the release of preformed LH, their participation in such cellular processes as hormone synthesis, packaging and movement of storage granules is not precluded.

It is evident that hormonal modulation of GnRH action could operate through alterations in any or all of these intracellular processes, either singly or in combination.

**Table 1. Cellular biochemical events involved in GnRH-stimulated LH release (established) (a) and other cellular processes activated by GnRH and their relationship to LH release/synthesis (uncertain) (b)**

| (a) | 1. GnRH binding to specific membrane receptors |
| 2. Ca²⁺ entry into gonadotroph/calcium activation |
| 3. LH release from preformed granules |
| (b) | 1. Elevation of cyclic AMP (conflicting literature) |
| 2. Elevation of cyclic GMP (not a prerequisite for LH release) |
| 3. Increased phospholipase A₂ activity (Ca²⁺-dependent) |
| 4. Microfilament function |
| 5. Processing of hormone–receptor complexes (independent of LH release) |
| 6. Gonadotrophin synthesis (Ca²⁺-independent?) |
Role of GnRH receptors in physiological regulation of gonadotropin secretion

With reliable methods for analysis of the GnRH—receptor interaction it has been possible to investigate steroid hormone modulation of this initial step in GnRH action.

With respect to ovarian steroid regulation of pituitary responsiveness, maximal GnRH receptor concentrations are found on the morning and early afternoon of proestrous, when pituitary responsiveness to GnRH is maximal. This increase in GnRH receptors on proestrous is maintained until the time of the spontaneous pre-ovulatory LH surge, when a dramatic fall, to nadir values, is consistently found [22–24]. The receptor fall might reflect increased endogenous GnRH secretion [25], with subsequent accelerated processing of hormone—receptor complexes [23], though this awaits confirmation. GnRH receptors in monkey pituitaries can also be increased by oestrogens, indicating that receptor changes might be relevant in regulation of primate gonadotrophin secretion [26]. In all circumstances where GnRH receptor concentrations vary, the changes cannot be accounted for by alterations in the receptor affinity [13, 22–26].

While the rodent oestrous cycle provides one example of a positive correlation between pituitary GnRH receptor concentration and enhanced pituitary responsiveness to GnRH, another is evident following gonadectomy. In both male and female rats, a rapid doubling of GnRH receptor concentration occurs following removal of gonadal steroid feedback (Table 2) [27–29].

Further evidence for the regulatory role of gonadal steroids on GnRH receptor concentration is provided by their analysis during sexual maturation. In male animals there is an inverse correlation between GnRH receptor values and the serum concentrations of testosterone (Table 2) [30].

During lactation, in rodents and primates, basal serum LH levels are very low, pulsatile LH secretion is absent and pituitary responsiveness to GnRH is much reduced. In this situation, pituitary GnRH receptor values are 50% below the lowest values recorded during the oestrus cycle [23].

The above physiological situations all demonstrate a positive correlation between basal serum LH values and pituitary GnRH receptor concentrations, implying a role for receptor regulation in determination of pituitary responsiveness.

GnRH regulation of its own receptors

Endogenous GnRH secretion is increased post-gonadectomy (GnRH receptors high), and is decreased during lactation (GnRH receptors low). Thus, endogenous GnRH itself might determine the concentration of its own pituitary receptors in vivo. This hypothesis has been tested by analysis of the GnRH receptor response to castration following elimination of endogenous GnRH secretion, or antagonism of GnRH action in the pituitary. The results summarized in Table 3) support the view that endogenous GnRH is essential for the post-castration increase in GnRH receptor concentration [31–34]. An important corollary to the concept of GnRH autoregulation of its receptors is that their measurement can provide a qualitative index of pituitary exposure to GnRH, and hence of hypothalamic function in vivo. Thus, low GnRH receptor levels in the lactating rat indicate reduced hypothalamic GnRH secretion, entirely consistent with the conclusions derived from measurement of serum gonadotrophin values.

The hypothesis of GnRH receptor autoregulation was substantiated in studies performed employing the classical physiological approach of ablation and replacement. Additional support for the theory comes from experiments in animals.
with an intact hypothalmo–pituitary–gonadal axis. Short-term intermittent injection of GnRH or a GnRH agonist analogue (GnRH-A) effects modest GnRH receptor increases [23, 28]. Conversely, in the hypogonadotrophic hypogonadal mouse (hpg), in which there is an inherited deficiency of GnRH secretion, the GnRH receptor concentration is only 30% of that of normal littermates [35].

From the foregoing, it is apparent that gonadal steroid regulation of pituitary GnRH receptor concentration might be secondary to their effects on endogenous GnRH secretion. However, steroids may also modulate GnRH receptor concentration by direct action on pituitary gonadotrophs [36].

Dependence of pituitary responsiveness upon mode of GnRH exposure

After an initial stimulation of gonadotrophin release, continuous pituitary exposure to GnRH, or agonist analogues, is followed by a progressive fall in hormone output. This 'stimulus-induced' pituitary refractoriness, or 'desensitization', cannot be explained by depletion of releasable hormone [12]. Possible mechanisms to explain pituitary refractoriness include (i) ligand-induced receptor loss (or 'down-regulation'), [37] and/or (ii) 'uncoupling' of occupied hormone–receptor complexes from subsequent intracellular events required for hormone release.

The mechanisms of pituitary refractoriness have been examined in animals infused continuously with various doses of GnRH. A dose-dependent reduction in free GnRH receptor concentration, pituitary LH content and basal serum LH values was observed [34]. With highest GnRH concentrations it was shown that reduction in GnRH binding could be accounted for by receptor occupancy (65%) in addition to net receptor loss (35%). Despite the high level of receptor occupancy, basal serum LH and pituitary LH values declined, indicating that occupied receptors are somehow 'uncoupled' from the hormone release machinery. Reduction in LH available for release also contributed to refractoriness and desensitization. A low pituitary LH content when many GnRH receptors are occupied indicates that functional GnRH receptors are also required to maintain hormone synthesis, in addition to release.

General principles of hormone action and therapeutic implications

The GnRH–pituitary gonadotroph system highlights several important principles in hormone action, which are directly relevant to their therapeutic usage.

Intermittent exposure of the gonadotroph to GnRH may induce receptors and amplify cellular responsiveness to subsequent stimulation. However, the ligand-inducing effect upon its receptors spans a narrow dose range above which the opposite effect of receptor 'down-regulation' and cell refractoriness is observed. This latter may represent a generalized cellular mechanism to restrict the effects of excessive stimulation. Thus, modification of the initial step of hormone action on a target cell forms an important regulatory mechanism in determining tissue responsiveness.

For long-term stimulation of gonadotrophin secretion, GnRH must be administered in low doses and with a frequency similar to that occurring naturally. In this manner low-dose pulses of GnRH, given 2-hourly, restore full gonadal function in patients with absence of endogenous GnRH secretion [38–41]. As in the hypothalamic-lesioned monkey [6], serum gonadotrophin and sex steroid profiles were strikingly similar to those of spontaneous
menstrual cycles [39] and the pattern seen during the peri-pubertal maturation of the pituitary–gonadal axis [38]. Prolonged pulsatile GnRH therapy can also induce normal spermatogenesis and fertility in males in selected instances. The practical problems of GnRH delivery for long-term treatment have been overcome by the ingenious conversion of light-weight motor driven syringes programmed to expel intermittent pulses of GnRH via subcutaneously or intravenously sited cannulae.

The desensitizing property of high-dose, frequent, administration of the long-acting GnRH agonist analogues can also be employed where suppression of gonadal activity is desired. In such circumstances the development of nasal sprays containing ‘slow-release’ formulations of the peptides are an obvious advance for patient compliance. By the nasal route long-acting GnRH analogues can successfully abolish menses and ovulation [11], though the reliability and suitability of this approach for widespread contraceptive usage remains to be determined. In males, spermatogenesis can be suppressed [42], but only at the expense of markedly reduced serum testosterone levels, with a concomitant unacceptable decrease in libido and potency. A ‘medical’ gonadectomy might be an acceptable alternative to surgery in the treatment of hormone-dependent tumours, e.g. mammary and prostatic carcinomas. Dramatic clinical and hormonal remission can be achieved by GnRH analogue treatment of true precocious puberty [43]. Other potential antigonadal therapeutic applications of long-acting GnRH analogues are in endometriosis and for severe premenstrual tension. It appears, therefore, that the paradoxical antifertility properties of GnRH peptides will have greater clinical application than the pro-fertility ones for which they were originally intended. A further important advantage of the antifertility property of GnRH analogues is its rapid reversibility [44] and freedom from long-term side effects.

References


