Review Article

Hypothalamo-Pituitary-Gonadal Axis in Control of Female Reproductive Cycle

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Abstract: Gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus is pivotal to the regulation of reproductive physiology in vertebrates. The characteristic periodic secretion of gonadotropin releasing hormone (GnRH) from the medial basal hypothalamus (MBH), at the rate of one pulse an hour is essential for the maintenance of the menstrual cycle. These pulses are due to oscillations in the electrical activity of the GnRH pulse generator in the MBH. The GnRH pulse generator is under the influence of an assortment of interactions of multiple neural, hormonal and environmental inputs to the hypothalamus. Hence, a number of conditions such as stress, drug intake, exercise, sleep affect the activity of this pulse generator. Any deviation of normal frequency results in disruption of normal cycle. The cycle can become anovulatory in the hypothalamic lesions and can be restored by exogenous administration of pulsatile GnRH.

Of late, studies have shown that pulse generator activity is also maintained by specific metabolic signals meant for energy homeostasis. Studies are in progress to work out cellular basis of GnRH pulse generator's rhythmic activation and role of Ca++ as second messenger for GnRH stimulated gonadotropin release. New concepts are emerging to find the existence of an FSH releasing factor, which independently regulates the activity of FSH.

Key words: GnRH pulse generator gonadotropins neuro-endocrine

INTRODUCTION

The regulation of the reproductive cycle in humans is under the influence of a range of neuro-endocrine processes (1,2,3). The main components of this system are the pituitary, the hypothalamus, which in cognizance with higher centers, brings about the required effect on the gonads. These are influenced by diverse, possibly opposing, external and internal environmental signals which are recognized, interpreted, collated and transduced into a specific neuro-humoral direction, which guide the brain and pituitary, to meet the body's changing physiological needs (1, 2, 4, 5, 6, 7). Neural
inputs from the limbic area, to the medial basal hypothalamus (MBH), have been quantified during estrous cycle (8) and further their interactions with the immune system has also been worked out (8). Over the past 4 decades, a number of concepts have evolved and that often have been modified, which has explained and strengthened the understanding of this complex interaction. Our data have shown that neural substrate in MBH regulates not only endocrine events, but also those involved in immune responses during an ovulatory estrous cycle (8, 12, 15). This is important for the clinical relevance, such as linking various psychological factors to structural, immunological or pathological abnormalities to the disruption of the axis. As a result GnRH analogues are currently being used for the treatment of prostate cancer, endometriosis, precocious puberty and induction of ovulation (20).

This article provides with a brief overview of the current concepts of neuro-endocrine involvement in the control of gonadotropin secretion as studied in recent years. This will be useful in the understanding of the complex hitherto mysterious mechanisms, such as stress, diet, exercise, and other diverse influences on the pituitary gonadal axis. We have mainly concentrated in discussing the structures involved in the control of the hypothalamo-pituitary axis.

Role of hypothalamo-pituitary axis in control of female ovarian cycle

The two major sites of action within the brain, which are important in the regulation of reproductive function, are the hypothalamus and the pituitary gland. Over the years the concept of pituitary as the master gland has given way to a new notion of the hypothalamus as the conductor, which responds to both peripheral and central nervous system messages and exerts its influence by means of neurotransmitters transported to the pituitary via the portal vessels (1, 2, 7, 9, 10). Whatever the regulating influence from the higher centers, the menstrual cycle is brought about by the sex steroids produced within the ovarian follicle. This is possible by the feedback mechanism of the sex steroids on the gonadotropins on the anterior pituitary (1, 2, 7, 9).

The hypothalamus acts as a central station where neural and hormonal messages arising from internal and external stimuli are decoded and the appropriate message sent to the anterior pituitary. As early as in 1930, Moore-Pierce theory attempted to explain the reciprocal influence between the gonads and the pituitary (7). The feedback principle was explained as the see-saw or the push-pull effect. Ernst Scharrer in 1928 proposed that the neurons have a secretory function, where the chemical nature of the neurohormones vary, ranging from biogenic amines to small peptides (1, 2, 7). Until the 1940's, the concept of the anterior pituitary as the master gland that controls the gonads, adrenal cortex, and thyroid held good. The role of external factors such as the day length on the breeding cycle in rabbits and preovulatory blockade of LH surge by certain neural blocking drugs were suggested by Marshall et al (7).
Anatomy of the hypothalomo-hypophyseal tract

The hypothalamus is situated at the base of the third ventricle, in the area between the optic chiasma anteriorly, and the mammillary bodies posteriorly. There is however a lack of clear understanding in the boundaries between the cellular nuclei of the hypothalamus (9). Anatomically the functional components are divided as:

(i) The hormones of the hypothalamic-neurohypophyseal tract (posterior pituitary): These are the direct neurosecretory fibers of the hypothalamus, discharging their secretions in the systemic blood. Vasopressin and oxytocin are synthesized in the paraventricular and the supra-optic nuclei of the hypothalamus and are transported via the hypothalamo-hypophyseal tract to the posterior pituitary (1, 2, 5).

(ii) The hormones for the anterior pituitary are secreted at the median eminence. They are then transported to the anterior pituitary via a specialized hypothalamic-pituitary portal system. Most of the hormones are hypothalamic releasing and/or inhibiting hormones (1, 2, 7, 9).

(iii) Numerous efferent and afferent pathways connect the hypothalamus (1, 2).

Median eminence: acts as a receiving station that funnels all the neuronal signals to the pituitary. The releasing hormones of the hypothalamus are deposited at the median eminence to be picked up by the portal vessels and ultimately transported to the anterior pituitary. On the other hand, the neurons from the pre-optic and suprachiasmatic nuclei traverse the median eminence to directly terminate at the posterior pituitary (1, 2).

Embryologic development of the hypothalamus and pituitary

1. The anterior pituitary develops as an outpouching of the foregut in the 5th week of the intrauterine life. This is called the Rathke's pouch which later migrates to join the posterior pituitary at the infundibular process (1, 11, 13).

2. The rostral GnRH-releasing cells migrate from the medial olfactory placode across the nasal septum and into the forebrain area (11). They migrate along the nervus terminalis to the hypothalamo-septal-preoptic area. They are present as a diffuse population of cells, which are essentially a loose continuum from the telencephalic diagonal band of Broca and the septal nuclei, through the bed nucleus of stria terminalis, to diencephalic areas (encompassing the medial, lateral and periventricular preoptic areas, anterior hypothalamus and retrochiasmatic zone). These neurons ultimately impinge on the Median Eminence and control the adenohypophyseal secretions (11).
Gonadotropin Releasing hormone (GnRH): (luteinizing hormone releasing hormone; LHRH).

Initially it was assumed that the secretion of each anterior pituitary hormone was controlled by the action of both releasing and inhibiting substances produced by the hypothalamus. Hence two hormones were believed to exist as the FSH-releasing hormone and LH-releasing hormone (14, 16). The hormone was later isolated and characterized as the luteinizing hormone releasing hormone. As this hormone also affected the secretion of FSH it was named as Gonadotropin Hormone releasing hormone (GnRH) (4, 17, 19).

The porcine GnRH was synthesized and characterized by Schally and Guillemin (1971). The gene sequence was isolated on chromosome 8 in 1984 by Seeburg et al (18,19). Chemically it is a decapeptide, derived from the post-translational modification of its pre-pro form. The neurons in the arcuate nucleus produce this hormone and secrete it in the median eminence (19). It is then transferred to the anterior pituitary, causing the production of FSH and LH.

In response to the GnRH stimulation the anterior pituitary produces LH and FSH. The LH production is prompt reaching the peak in 5 minutes and FSH peak in 30 minutes (7, 9). GnRH has a very short half-life (2–4 minutes) making it very difficult to measure its levels in the serum. The levels can however be estimated indirectly by measuring the serum levels of LH (9). In the experimental set up the GnRH estimation is possible by the push-pull cannula method, by sampling the hypophyseal portal blood. These experiments have been carried out on rats and the rhesus monkeys (22).

GnRH neuronal system

In mammals the GnRH neurosecretory system morphologically consists of a diffuse network of about 800–2000 functionally connected neurons (13,23). The characterization of these neurons is done using the immuno-cyto-chemical technique, by staining the GnRH-immunoreactive neurons. The GnRH neurons are typically oval or fusiform in shape; with simple, unbranched dendritic processes from one or both poles of the cell (13). Axons emerge either directly from the cell body or from a dendrite. These neurons have a large, centrally placed nucleus and a thin cytoplasm. The cytoplasm contains many stacks of rough endoplasmic reticulum, Golgi bodies, and neurosecretory granules. The neurons show a typical pulsatile secretion, this is typically species specific of a frequency of 20 min to 2 h, which is essential for the expression of the hormone (5, 13, 17, 24).

Physiological features:

a. Pulsatile and intermittent secretion: the mean level of the hormone varies through the menstrual cycle. A unique feature about the hormone is a circhoral secretion of one pulse per hour. This pattern of secretion is responsible for the physiological effects of the hormone (26, 28, 29, 31, 34).
b. Continuous secretion causes desensitization and complete refractoriness of the pituitary gland to gonadotropin secretion leading to gonadal suppression (6, 25, 28, 30).

c. If the frequency of the pulses are not in the physiological range of one pulse per hour, normal gonadotropin hormone secretion cannot occur. Any increase or a decrease will abolish the gonadotropin secretion (27, 28, 32, 33).

d. If the frequency of GnRH is re-established in the physiological range of one pulse per hour, normal 28-day menstrual cycles begin (28, 32).

e. Developmental pattern: another feature of this hormone is that it shows a developmental pattern in its secretion. In the infants, the gonadotropin secretion is active during the neonatal period, as early as the 55 day of the intra-uterine life. As the foetus matures, the activity of the GnRH secreting neuron decreases continuously. In the infancy, the secretion is almost quiescent. This continues until the adolescent period. Thereafter a reemergence of the GnRH activity is observed, (28, 31) until the menopause wherein the activity completely ceases. The fine tuning of the GnRH neurons occurs at the birth, puberty and at menopause (28).

Hypothalamic-pituitary-ovarian interactions

Menstrual cyclicity and timely ovulation are the result of precise integration of a series of events occurring within the different components of the reproductive system (17, 26). This integrity takes place at three levels. (i) The activity of the GnRH pulse generator, (ii) The pituitary secretion of gonadotropins and (iii) The estradiol positive feedback for the pre-ovulatory LH surge, oocyte maturation and corpus luteum formation (18).

The GnRH pulse generator: When the GnRH secreting neurons are deafferentiated, i.e. isolated from all the influences of the anterior pituitary and the higher centers, the neurons have a frequency of approximately one discharge per hour (28, 29, 34). This causes a release of a bolus of GnRH into the pituitary portal circulation. The pituitary gonadotropes respond to each GnRH pulse by discharging a pulse of LH and FSH into the peripheral circulation. During the follicular phase of menstrual cycle the GnRH pulse generator functions as in the unmodulated state (28, 29). However, the rise in the levels of estrogen in the follicular phase has a feedback inhibitory control over the anterior pituitary. The peripheral levels of estrogen can also alter the activity of the GnRH neurons by a direct or an indirect action (23). Recent evidence suggest the
involvement of neurotransmitters like catecholamines, gamma-aminobutyric acid, glutamate, neuropeptide Y, neurotensin, β-endorphin and vasoactive intestinal polypeptide on the GnRH neurons (23). It is suggested that estradiol influences the activity of these neurotransmitters with in the GnRH network to drive the reproductive cycle (23).

The continued stimulation of the anterior pituitary by the hypothalamus and its inhibition by the feedback mechanism by circulating estrogen creates a balance and the net effect is an increase in the estrogen levels (1, 12, 28, 29). Hence, in the follicular phase the estrogen levels continuously rise until a threshold level is achieved (~250 pg/ml for >36 hours) (18, 28, 29). At this level, the inhibitory (negative feedback) effect of estradiol is suddenly reversed and the pituitary discharges the pre-ovulatory surge of LH and FSH (positive feedback) (Fig. 1). The GnRH pulse frequency or the amplitude is not a prerequisite for this LH surge (28, 29). The ovaries respond to gonadotropin LH surge by ovulation and a sharp rise in the levels of estrogen and progesterone (1, 2, 9, 28, 29).

The GnRH pulse generator activity is affected by the variation in the photoperiod stimulation via the pineal melatonin. Administration of melatonin in the MBH reactivates the GnRH synthesis. (33). Melatonin however also acts directly on the pituitary to produce prolactin. Thus in the animals it is responsible for the breeding cycles associated with the seasonal variations (33). The availability of the energy in the environment also modulates the activity of the hypothalamic-pituitary-gonadal axis (31). It function is dependent on the availability of abundant energy in the environment, any modification in energy balance causing a tilt in the energy balance, suppressing the activity of the GnRH pulse generator. Leptins play an important role in control of this balance (31).

Location of the pulse generator: Autonomous secretion of GnRH is seen in isolated hypothalamic nuclei and the activity is most prominent in the Medio-basal hypothalamus, which corresponds to the Arcuate Nucleus (24). Knobil et al (28, 29) found that continuous stimulation of the medio-basal nucleus caused a decrease in the gonadotropin release. They also recorded the electrical activity of this area and observed a sudden increase in the multiunit electrical activity in the medio-basal hypothalamus, which were coincident with the initiation of the LH pulse in the peripheral circulation (30, 34). He further noticed that progesterone reduced the frequency of the pulse generator as seen in the luteal phase (28, 29).

Mechanism of action of GnRH: The GnRH has its receptors on the pituitary gonadotrophs. After binding to the specific receptor, it is
internalized and undergoes degeneration by the lysosomes. (21, 37, 38, 39). The receptor fragments are rapidly recycled. An intermittent secretion of GnRH, thus maintains the activity of the receptor by continuous recycling. However, on continuous exposure to GnRH it produces suppression of LH release, by reducing the number of GnRH receptors. (38, 39).

The binding of the GnRH to its receptor complex produces a complex series of intracellular responses, which result in secretion and biosynthesis of the α and β subunits of LH and FSH (38, 39). Within seconds of GnRH binding to the GnRH receptors (GnRHR) on the pituitary gonadotrophs, intracellular free Ca²⁺ concentration increases (41). This Ca²⁺ is initially derived from the endoplasmic stores, but extracellular Ca²⁺ enters the cell through receptor-regulated voltage dependent Ca²⁺ channels. (42) G-proteins are stimulated which cause the activation of membrane bound phospholipase C, which in turn activates inositol triphosphate and diacyl glycerol. Inositol triphosphate mobilizes Ca²⁺ ions and diacyl glycerol activates phosphorylating enzyme protein kinase C (38, 39). Adenyl cyclase is also stimulated and cAMP is generated. Ca²⁺ ions, protein kinase C and cAMP all then interact to synthesize and stimulate release of stored LH and FSH (2, 38, 39).

Knobil (28) suggested for a normal menstrual cycle an unvarying hourly pulsatile secretion of GnRH is adequate to obtain the normal 28 day cycle. This secretion is in turn controlled by the levels of estrogen and progestrogen from the ovary. However, the progestrogen from the corpus luteum reduces the frequency of the pulse generator, but it does not affect the cyclicity of the menstrual cycle as normal cycles can be maintained by a constant stimulation of the GnRH neurons either by electrical stimulation or by infusion of hourly GnRH (28, 29).

Other important features noted by Knobil were (28, 29):

- **GnRH at reduced pulsatile frequency**: One pulse every 3 hours leads to increase in mean plasma FSH and decrease in mean LH concentration in the blood.
- **At lower pulses the follicular development continued albeit with a lower incidence of ovulation compared with the controls.**
- **Administration at every 90 minute permitted initial ovarian cycle, but subsequent cycles did not culminate in ovulation** (29).
- **The basal LH secretion was important for the normal follicular development.**
- **Small changes in the GnRH stimulation have profound effects on the quality of follicular development and any alteration in the frequency of endogenous GnRH release, occasioned by higher neural centers or other factors, may play a major role in the control of ovarian function, including the induction of puberty** (29).
In ovarectomised monkeys, a characteristic increase in the circulating gonadotropins is seen, which reaches steady state in approximately 3 weeks. This pattern is pulsatile and is similar to the one in the non-ovarectomised state albeit levels are high and is at the rate of 1 per hour. This pattern can however be abolished by introduction of physiologic concentration of estrogen. Further the administration of progestrogen does not cause any effect on the gonadotropin secretion.

Hence, estradiol is the principal ovarian hormonal component, which governs the tonic gonadotropin secretion (43, 44).

The GnRH Receptor: Hormonal signaling in the reproductive axis requires intact receptors for appropriate message transduction. GnRH signals through the G protein-coupled receptors (GPCRs). Abnormalities in these receptors, or in the intracellular downstream transduction system, results in either enhanced or inhibited gonadal function (38, 45).

**Fig. 2:** Mechanism involved in GnRH action on the gonadotrophs.
The GnRH receptor (GnRHR) has a seven transmembrane domain structure with an extracellular amino terminus and a truncated cytoplasmic tail at its intracellular carboxyl end. The receptor requires intracellular guanine nucleotide binding (G) proteins (Gq/G11) to turn on the effector enzyme phospholipase C. This in turn, enhances inositol triphosphate (IP) production and increases intracellular calcium, which ultimately leads to the synthesis and secretion of the gonadotropins (Fig. 2). The human GnRHR gene has three exons and is located on chromosome 4. The principal location of the receptor is on the cell surface of pituitary gonadotropes (38). These cells, located in the anterior pituitary, respond to GnRH with the synthesis and secretion of FSH and LH, which mediate gonadal steroidogenesis and the development of secondary sex characteristics and fertility (38, 40, 45).

The defects in the mutated GnRH receptor may lead to diminished ligand binding and/or altered receptor transduction efficiency. However, no human mutations lead to constitutive or enhanced GnRHR activity. Agonist stimulation provokes a conformational change in the receptor molecule to allow a signal transduction. This is an efficient process and involves a tertiary complex containing receptor, G-protein, and effector molecules (Phospholipase C and adenyl cyclase), that function optimally when each protein interacts with its neighbor at a specific site. These interactions are finally controlled by the conformation of each component molecule. Any variation in the amino acid sequence, by insertion, deletion or change in the sequence will result in altered function (38, 45).

The other areas where the GnRHR have been found are in the hippocampus, in Leydig and granulosa cells, hypothalamus and adenohypophysis. The current literature does not mention the extra pituitary effects of the hormone (45).

Interactions with other neurotransmitters

1. Monoamines:

(a) Dopamine: has contradictory role. The hypothalamic tuberoinfundibular dopaminergic pathway is formed by these neurons, with cell bodies located in the arcuate nucleus. The axons project to the median eminence. When the pituitary cells are coincubated with the hypothalamic fragments and if dopamine was added the release of LH increased, addition of phentolamine a blocker prevented the dopamine induced LH release. However, in vivo it has an inhibitory role as seen in hyper prolactenemia where there is an increase in dopamine which may be responsible for the gonadal suppression (1, 23, 44).

(b) Noradrenaline: most experimental evidence supports a stimulatory role of NA in controlling the gonadotropin release. The levels of NA are increased prior to the
preovulatory surge of the gonadotropins. Selective blockade of NA synthesis prevents the preovulatory LH surge. There is evidence that the NA fibers synapse with preoptic and anterior hypothalamic nuclei (1, 13, 23). However, there seems to be a differential effect of intraventricular LHRH and NE on arcuate nucleus, suggesting ultra-short loop negative feedback affect. (57)

c) Serotonin: High concentration of serotonin is found at the median eminence, with most of the serotonin containing neurons originating from the raphe nucleus in the mid-brain-pons area. Serotonin plays predominantly a inhibitory role (1, 13, 23).

2. Endogenous opioids: These have predominantly an inhibitory role in the GnRH secretion. In the in-vitro experiments, a single dose of morphine administered to monkeys brings about immediate cessation of the GnRH pulse generator (43, 46). Administration of the opioid antagonist (Naltrexone) produces an increased frequency and amplitude of GnRH and LH secretion. (10, 43). Various stresses in the form of mechanical, physiological or immunological insult result in increase of corticotropin releasing factor (CRH), which activates the hypothalano-pituitary-adrenal axis. CRF can also directly act on GnRH neurons causing down regulation of GnRH synthesis via endorphin release. (3, 23, 46)

3. Prostaglandin: The role of prostaglandin is not very clear. The brain synthesizes and releases prostaglandin's. Only the Prostaglandin E is important and can induce the release of GnRH. (1, 23)

The FSH releasing hormone?

Padmanabhan et al (47) have suggested the presence of an FSH releasing factor in the hypothalamus. This is different from the GnRH and causes the release of FSH. The hormone has not yet been characterized, but an overwhelming evidence for its existence is present.

The earlier contention that the LH and FSH have a single releasing hormone has recently been questioned. A new hypothesis has been put forth by a number of authors claiming a separate releasing factor for FSH (47, 48, 49, 50, 51, 52, 53, 54). This has at present been labeled as a FSH-releasing factor as the characterization has not yet been carried out and much of the hypothesis is a speculation (47).

Determining the nature of FSH releasing factor presents as a challenge because it has a long half-life and has molecular heterogeneity (not all isoforms of FSH have been recognized) (47, 48).

FSH release: (Basal and Episodic release). In contrast to the absolute dependence of LH secretory system on GnRH pulsatility, the FSH secretion has a
basal (constitutive) and a episodic (regulated) release. Substantial release of FSH is dependent on the constitutive release (26, 24, 49, 55, 56). A small portion of this secretion is however episodic and is independent of GnRH, (48) this accounts for about 1/3 of the total FSH thus released. Culler et al (13) have demonstrated that by combined administration of GnRH antiserum and GnRH antagonist abolished the LH pulses without affecting the FSH pulses. All these findings indicate the presence of FSH releasing factor.

Conclusion

The male and female pattern of gonadotropin and gonadal secretion is determined by different hypothalamic mechanisms. GnRH, secreted by neurons of the hypothalamus into the portal system, controls the release of both FSH and LH, which in turn secrete estrogens and progestrogens. For physiological effects, circchoral GnRH secretion is required. When the frequency is decreased (<1/hour), it results in suppression of ovulation and prolonged exposure to GnRH also leads to annovulation by down regulation of the receptors. Ovarian steroids help in modulation of GnRH production. Other factors are also involved in such regulation. Although the GnRH pulse generator plays an important role in controlling the reproductive cycle and its activity can be identified electrophysiologically, very little is known as to (i) what actually establishes the frequency/amplitude of pulses or in other words, which area in the brain is responsible for the pacemaker activity of the GnRH cells. (ii) How frequency is modulated and how is duration of activation determined?

Recent evidence has shown that FSH is under the influence of a separate releasing factor, it is tentatively known as the FSH, releasing factor. However, its existence is still speculative as the characterization of this molecule has still not been done.

REFERENCES


