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*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-pituatry 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 feed back 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 hypothalamo-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 pituatry) : These are the direct neurosecretory fibers of the hypothalamus, discharging their secretions in the systemic blood. Vasopressin and oxytocin are synthesized in the para-ventricular 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 supra-chiasmatic 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 a outpouching of the foregut in the 5$^{th}$ 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 in to 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).

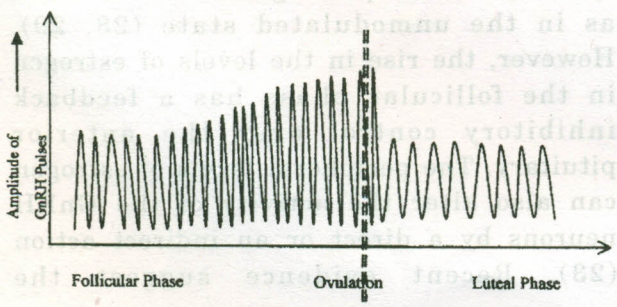


Fig. 1 : Schematic representation of the pulsatile secretion of GnRH during the menstrual cycle.

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 form the endoplasmic stores, but extracellular Ca2+ enters the cell through receptor-regulated voltage dependent Ca 2+ channels. (42) G-proteins are stimulated which cause the activation of membrane bound phospholipase C, which inturn activates inosityl triphosphate and diacyl glycerol. Inosityl triphosphate mobilizes and 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 effect the cylicity 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).

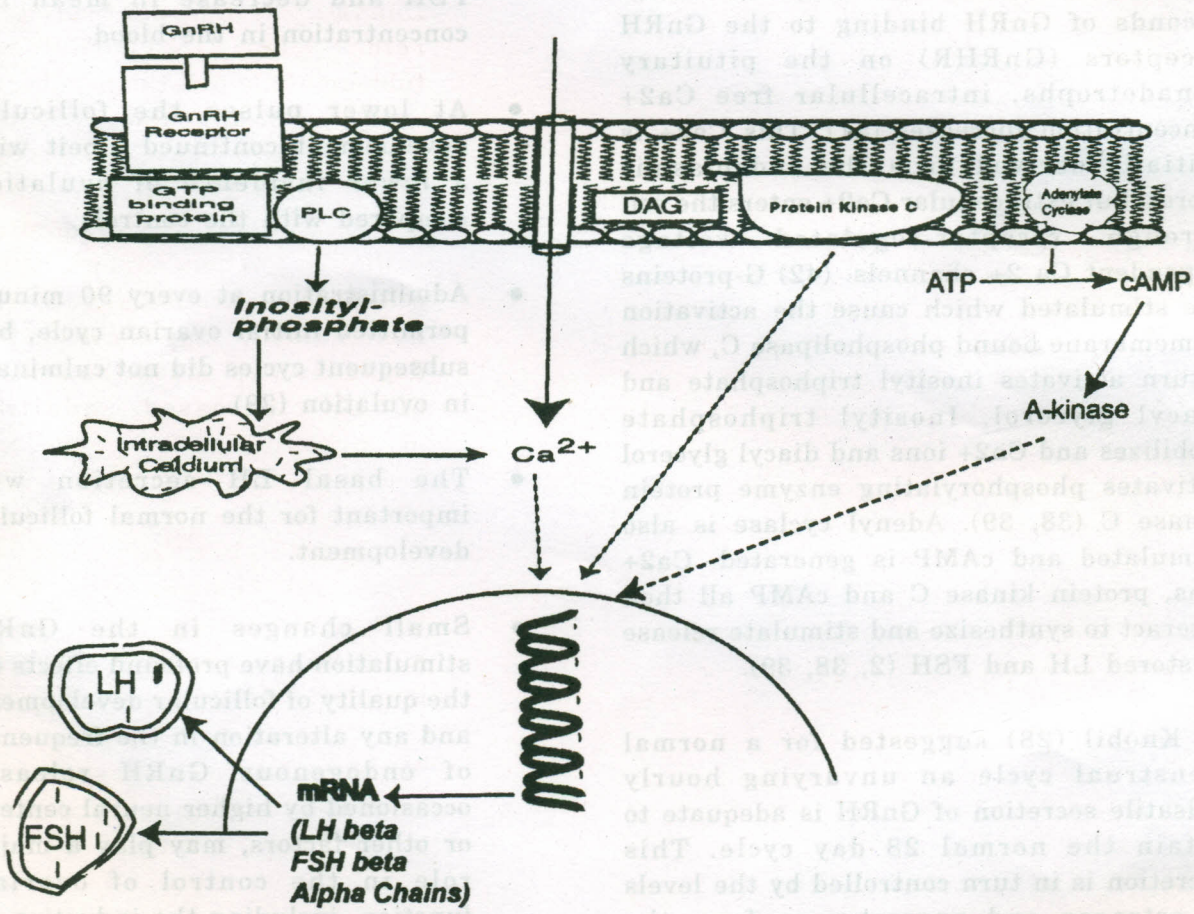


Fig. 2 : Mechanism involved in GnRH action on the gonadotrophs.

The GnRH receptor (GnRHR) has a seven transmembrane domain structure with an exracellular amino terminus and a truncated cytoplasmic tail at its intracellular carboxyl end. (38) The receptor requires intracellular guanine nucleotide binding (G) proteins (Gq/G11) to turn on the effector enzyme phospholipase C. This is 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 steroidoenesis 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 to 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 menion the extra pituitary effects of the hormone (45).

**Interactions with other neurotransmitters**

1. Monoamines :

(a) *Dopamine :* has contradictory role. The hypothalamic tubheroinfundibular 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 a increase in dopamine which may be responsible for the gonadal suppression (1, 23, 44).

(b) *Noradrenaline :* most experiemental 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 intra-ventricular 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 a 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 opiod antagonist (Naltrexone) produces an increased frequency and amplitude of GnRH and LH secretion. (10, 43). Various stresses in the form of mechanical , physiological or immunilogical insult result in increase of corticotropin releasing factor (CRH), which activates the hypothalamo-pituitary-adrenal axis. CRF can also directly act on GnRH neurons causing down regulation of GnRH synthesis via β 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 pulsatality, 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, circhoral 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


1. Shaw RW. Control of hypothalamic-pituitary ovarian function; Gynecology 2nd edn, Shaw RW, Soutter PW, Stanton SL (Eds). Churchhill Livingstone 1997; 171–183.
2. Gordon K, Oehninger S. Reproductive Physiology. *Text Book of Gynecology* 2nd Edn; Copeland LJ, Jarell JF (Edrs). W B Saunders Company 1993; 59–63.
3. Tellam DJ, Yasmin NM, Lovejoy DA. Molecular integration of hypothalamo-pituitary-adrenal axis-related neurohormones on the GnRH neuron. *Biochem Cell Biol* 2000; 78: 205–216.
4. Chappel SC. Neuroendocrine regulation of luteinizing hormone and follicle stimulating hormone: a review. *Life Sciences* 1985; 36: 97-103.
5. Everett JW. Central Neural Control of Reproductive functions of the Adenohypophysis. *Physiol Rev* 1964; 44 (3): 373–431.
6. Knobil E. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* 1980; 36: 53–88.
7. Yen SSC. The human menstrual cycle: neuroendocrine regulation: Yen SSC, Jaffe RB(eds) : *Reproductive Endocrinology 3rd Edn.* Philadelphia: WB Saunders Company 1991. 273
8. Tandon OP, Akema T, Kawakami M. Medial pre-optic unit responses to stimulation of medial amygdala and dorsal hippocampus during different phases of estrous cycle in the rats. *Proc Indian Nat Sci Acad* 1982; B49: 244–248.

9. Speroff L, Glass RH, Kase NG. Newuroendocrinology. *Clinical Gynecologic Endocrinology and Infertility 3rd Edn.* Williams and Wilkins Co. Baltimore, Maryland, USA 1984; 41–75.

10. Wildt L, Leyendecker G. Induction of ovulation by the chronic administration of naltrexone in hypothalamic amonorrhoea. *Clin Endocrinol Metab* 1987; 64: 1334

11. Schwanzel-Fukuda M, Blick D, Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. *Nature* 1989; 338:161

12. Tandon OP, Electrophysiological correlation of neuroendocrine and immune responses in proestrous rats. *Annals NY Acad Sci* 1992, 650: 68–73.

13. Bahr MJ. The Neuroendocrine System; Gold JJ, Josimouch JB. *Gynecological Endocrinology* 4th Edn. Plenum Publishing Corporation, NY. 11–22.

14. Scally AV, Arimura A, Kastin AJ. Hypothalamic regulatory hormones. *Science* 1973; 179: 341.

15. Tandon OP, Kawakami M, Akaema T, Ohno S. Medical pre-optic responses to stimulation of limbic and brainstem areas in proestrous rats. Neurosciences 1982, 7 supp 320–327.

16. Scally AV, Arimura A, Kastin AJ. Gonodotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle stimulating hormones. *Science* 1971b; 173: 1036.

17. Crowley WF Jr, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Recent Prog Horm Res* 1985; 41: 473–531.

18. Handelsman DJ, Swerdloff RS. Pharmacokinetics of gonadotropin-releasing hormone and its analogs. *Endocr Rev* 1986; 7: 95–105.

19. Weiss J, Crowley WF Jr, Jameson JL. Normal structure of the gonadotropin-releasing hormone (GnRH) gene in patients with GnRH deficiency and idiopathic hypogonadotropic hypogonadism. Clin *Endocriol Metab* 1989; 69: 299–303.

20. Conn MP, Crowley WF. Gonadotropin-Releasing Hormone and its analogues. *N Engl J Med* 1991; 324(2): 93–103.

21. Conn Mp, Staley D, Harris C, Andrews WV, Huckle WR. Mechanism of action of gonadotropin releasing hormone. *Ann Rev Physiol* 1986; 48: 495–513.

22. Levine JE, Pau KY, Ramirez VD, Jackson GL. Simultaneous measurement of leutinizing hormone-releasing hormone and leutinizing hormone release in un-anesthetized and ovarectomized sheep. *Endocrinol 1982;* 111: 1449–55.

23. Smith MJ, Jennes L. Neural signals that regulate GnRH neurons directly during the estrous cycle. *Reproduction* 2001; 122(1): 1–10.

24. Sheridan R, Loras B, Rivier J Pasteels JL. Autonomous secretion of follicle stimulating hormone by long-term organ cultures of rat pituitaries. *Endocrinol* 1979; 104: 198–204.

25. Belchetz PE, Plant TM, Nakai Y Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 1978; 202: 631–3.

26. Clarke IJ, Cummins JT. The temporal relation between gonadotropin releasing hormone (GnRH) and leutinizing hormone (LH) secretion in ovarectomized ewes. *Endocrinol* 1982; 111: 1737–9.

27. Filicori M, Santoro N, Merriam GR, Crowey WF Jr. Characterization of the physiological pattern of episodic gonadotropin secretion throughput the human menstral cycle. *J Clin Endocrinol Metab* 1986; 62: 1136–1144.

28. Knobil E. Hypothalamic Pulse generator governs mammalian reproduction. *News in Physiol Sci* 1987; Vol 2: 42–43.

29. Knobil E, Plant TM. Neuroendocrine control of Gonadotropin secretion in the female Rhesus monkey. *Frontiers in Neuroendocrinology,* Vol 5, Raven Press, New York 1978.249–264.

30. Phol CR, Richardson DW, Hutchison JS, Germak JA, Knobil E. Hypophysiotropic signal frequency and the functioning of the pituitary-ovarian system in the Rhesus Monkey. *Endocrinol* 1983; 112(6): 2076–2080.

31. Lincoln G. Melatonin modulation of prolactin and gonadotrophin secretion. Systems ancient and modern. *Adv Exp Med Biol* 1999; 460: 137–153.

32. Waldhauser F, Weibenbacher G Frish H, Pollack A. Pulsatile secretion of gonadotropins in early infancy. *Eur J Pediatr* 1981; 137: 71–74.

33. Caprio M, Fabbrini E, Isidori AM Aversa A, Fabbri A, Leptin in reproduction. *Trends Endocrinol Metab* 2001; 12(2): 65–72.

34. Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E. Frequency and amplitude of GnRH stimulation and gonadotropin

secretion in the rhesus monkey. *Endocrinol* 1981; 109: 376–385.

35. Wilson RC, Kesner JS, Kaufman JM. Central electrophysiological coorelates of pulsatile luteinizing hormone secretion in the rhesus monkey. *Neuroendocrinol* 1984; 39: 256.
36. Jakacki RI, Kelch RP, Saunder SE, Loyd JS, Hopwood NJ, Marshall JC. Pulsatile secretion of Leutinizing hormone in children. *J Clin Endocrinol Metab* 1982; 55: 453–458.
37. Brown P, McNeilly AS. Transcriptional regulation of pituitary gonadotropin subunit genes. *Rev of Reprod* 1999; 4: 117–124.
38. Conn PM, Huckle WR, Andrews WV, McArdle CA. The molecular mechanism of action of gonadotropin releasing hormone (GnRH) in the pituitary. *Recent Prog Horm Res* 1987; 43: 29–68.
39. Huckle W, Conn PM. Molecular mechanism of gonadotropin releasing hormone action. II. The edffector system. *Endocr Rev* 1988; 9: 387–95.
40. Marshall JC, Dalkin AC, Haisenleder DJ, Griffin ML, Kelch RP. GnRH pulses-the regulators of human reproduction. *Transactions of the American Clinical and Climatological Association* 1992; 104: 31–46.
41. Marian J, Conn PM. Gonadotropin releasing hormone stimulation of cultured pituitary cells requires calcium. *Mol Pharmacol 1979;* 16: 196–201.
42. Hansen JR, McArdle CA, Conn PM. Relative roles of calcium derived form intra- and extracellular sources in dynamic leuitinizing hormone release from pituitary cells. *Mol Endocrinol* 1987; 1: 808–815.
43. Besch NF, Smith CG, Besch PK, Kaufman RH. The effect of marihuana (delta-9-tetrahydrocanabinol) on the secretion of lutinizing hormone in the ovarectomized rhesus monkey. *Am J Obstet Gynecol* 1977; 128: 635.
44. Richardson DW, Goldsmith LT, Pohl CR, Schallenberger E, Knobil E. The Role of Prolactin in the regulation of the Primate Corpus Luteum. *J Clin Endocrinol Metab* 1985; 60(3): 501–504.
45. Cohen DP. Molecular Evaluation of the Gonadotropin-Releasing hormone receptor. *Seminars in Reprod Med* 2000; 18(1):11–16.
46. Smith CG, Besch NF, Smith RG, Besch PK. Effect of tetrahydrocannabinol on the hypothalamic pituitary axis in the ovarectomized Rhesus monkey. *Fertil and Sterilit* 1979; 31: 335–339.
47. Padmanabhan V, McNeilly AS. Is there an FSH-releasing factor ? *Reproduction* 2001; 121: 21–30.
48. Padmanabhan V, McFadden K, Mauger DT, Karsch FJ, Midgely AR. Neuroendocrine control of FSH secretion I. Direct evidence for separate episodic and basal components of FSH secretion. *Endocrinol* 1997; 138: 424–432.
49. DePaolo LV, Bisak TA, Erickson GF, Shimasaki S, Ling N. Follistatin and activin: a potential intrinsic regulatory system within diverse tissues. *Proc of the Society for experimental Biology and Med* 1991; 198: 500–512.
50. Savoy-Moore RT, Schwartz NB. Differential control of FSH and LH secretion. *International Review of Physiol* 1980; 3: 65–68.
51 Thomas SG, Clarke IJ. The positive feedback action of estrogen mobilizes LH-containing but not FSH-containing secretory granules in ovine gonadotoropes. *Endocrinol* 1997; 138: 1347–1350.
52. McNeilly AS. The control of FSH secretion. *Acta Endocrinologica Supple* 1988; 122: 287–292.
53. Lescheid DW, Terasawa E, Abler LA, Urbanski HF, Warby CM, Millar RP, Sherwood NM. A second form of gonadotropin-releasing hormone (GnRH) with characteristics of chicken GnRH-II is present in the primate brain. *Endocrinol* 1997; 138: 5618–5629.
54. Dhariwal APS, Nallar R, Batt M, McCann SM. Seperation of follicle-stimulating hormone-releasing factor from luteinzing hormone-releasing factor. *Endocrinol* 1965; 76: 290–294.
55. Culler MD, Negro-Vellaar A. Pulsatile follicle stimulating hormone secretion is independent of luteinizing hormone releasing hormone (LHRH): pulsatile replacement of LHRH bioactivity in LHRH- immunoneutralized rats. *Endocrinol* 1987; 120: 2011–2021.
56. McCann SM, Mizunuma H, Samson WK, Lumpkin MD. Differential hypothalamic control of FSH secretion: a review. *Psychoneuroendocrinol* 1983; 8: 299–308.
57. Krieg RJ, Tandon OP, Whitmoyer DI, Sawyer CH. Differential effects of intraventricular Luteinizing hormone releasing hormone (LH-RH) and Norepinephrine on electrical activity of the arcuate nucleus in the proestrous rat. *Neuroendocrinology* 1976; 22: 152–163.