CHAPTER

Function of gonadotropin releasing hormone and inhibin

8

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Gonadotropin releasing hormone

Neuroanatomy

The hypothalamus is located at the base of the brain and forms the floor of the third ventricle. Neuroendocrine cells, which share characteristics of both neurons and endocrine glands, are located in the hypothalamus and respond to signals including neurotransmitters and subsequently produce and release hormones into the bloodstream or neural synapse.

Gonadotropin releasing hormone (GnRH) producing neuroendocrine cells arises from the olfactory placode of the developing brain. These cells migrate during embryogenesis through the olfactory bulb and forebrain to their final location in the arcuate nucleus of the hypothalamus, projecting toward the median eminence [1,2]. The number of GnRH releasing neurons in humans is between 1000 and 1500 [3]. The median eminence is surrounded by a robust capillary network known as the hypophyseal portal system which links the hypothalamus to the anterior pituitary. Impaired migration of the GnRH neurons is characterized by a physiological impact both on the olfactory area, represented by anosmia, and impaired GnRH secretion seen in Kallmann syndrome (Fig. 8.1).

GnRH structure

GnRH is a neuroendocrine releasing hormone responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. GnRH is initially synthesized and secreted by the GnRH neurons within the hypothalamus into the superior hypophyseal capillary network, which downstream mediates control of the gonadotropic production cells in the anterior pituitary. This is the initial step in the hypothalamic-pituitary-gonadal axis. The neurons exist in a clustered network of other cells allowing for close interaction with secreted neurotransmitters and hormones.

GnRH is a small tropic peptide hormone composed of 10 amino acids (Fig. 8.2). It was initially discovered in 1971 [4–6]. The structure of GnRH is largely conserved across mammalian species, however, the Tyr-Gly-Leu-Arg segment is variable,

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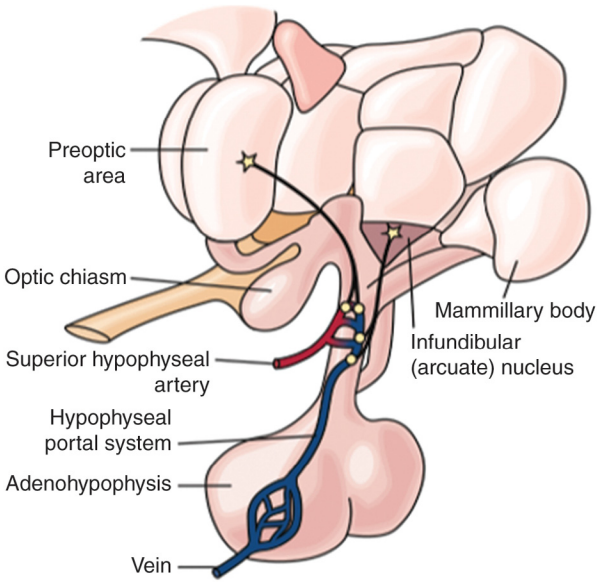

FIGURE 8.1

Anatomic relationship between GnRH neurons and their target cells in the anterior pituitary. GnRH neurons are located in the preoptic area of the hypothalamus. GnRH neuron projections (dendrons) then terminate at the median eminence, where GnRH is secreted into the hypophyseal portal system.

(Credit: Modified from Johnson MH, Everitt BJ: Essential Reproduction, ed 5. Blackwell, MA, 2000, Blackwell Science, Fig. 6.4. Credit: Yen & Jaffe) [38].

FIGURE 8.2

Structure of GnRH**.** GnRH is a decapeptide composed of 10 amino acids in sequence below.

although preserved in most vertebrate species [7]. The encoding gene for pro-GnRH, the precursor protein for GnRH, is located on Chromosome 8 (8p11.2-p21) and contains four exons and three introns encoding 92 amino acids [8]. Pro-GnRH contains a signal sequence of 23 amino acids followed by the 10 amino acid unit of GnRH. This is followed by a 3 amino acid proteolytic processing site and a final GnRH-associated peptide of 56 amino acids.

$$\mathbf{pGlu^{1}\text{-}His^{2}\text{-}Trp^{3}\text{-}Ser^{4}\text{-}Tyr^{5}\text{-}Gly^{6}\text{-}Leu^{7}\text{-}Arg^{8}\text{-}Pro^{9}\text{-}Gly^{10}\text{-}NH_{2}}$$

A second form of GnRH (GnRH-II) has been identified in many animal species, which has also been isolated in humans. The gene that encodes GnRH-II is located on Chromosome 20 (20p13) [9]. GnRH-II is not the predominant form found in the hypothalamus and is typically found in other organs. GnRH receptors have been found in the placenta, gonads, as well as breast, endometrial, epithelial, and stromal ovarian cancers although its purpose is not well understood [10,11].

GnRH secretion

GnRH is secreted into the vast capillary network of the hypophyseal portal system. It is quickly degraded once secreted and has a half-life of approximately 2–4 min. GnRH activates the GnRH receptor (GnRH-R), a G protein-coupled receptor with a seven-transmembrane domain. The gene for the receptor is located on Chromosome 4 and codes for the 328-amino acid protein [12]. After binding, phosphatidylinositol-4-5-bisphosphate (PIP2) is cleaved into second messengers diacylglycerol (DAG) and inositol triphosphate (IP3), IP3 stimulates intracellular calcium release. DAG, IP3, and calcium stimulate Protein Kinase C and the mitogen activated protein kinase (MAPK) cascades leading to increased transcription factors for gonadotropin production [13].

Gonadotrope responsiveness to GnRH secretion is modulated based on pulsatility of GnRH release. Neuronal activity is characterized by intrinsic short pulsatile patterns that change based on releasing and inhibiting factors including stress, energy availability, sex steroids, and neurotransmitters [14]. The pulsatility of GnRH is posited to emanate from the neurons in the arcuate nucleus of the hypothalamus [15]. Two modes of GnRH secretion have been identified: pulsatile and surge. Pulsatile secretion is mainly responsible for daily intermittent secretion of GnRH. Surge secretion is responsible for the pre-ovulatory LH surge that immediately precedes ovulation (Fig. 8.3). The pulsatile intermittent release maintains receptor reactivity to the presence of GnRH; however, increasing frequency of GnRH release may reduce

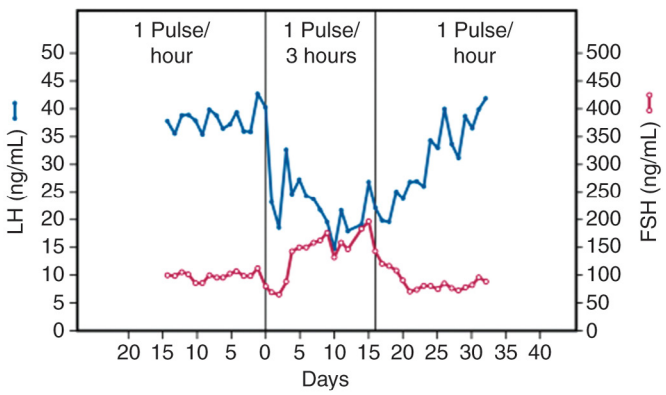

FIGURE 8.3

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations in gonadectomized (but not steroid-replaced) monkeys after arcuate nucleus ablation—a model of isolated GnRH deficiency. Exogenous GnRH administered in a pulsatile fashion every hour reconstituted LH and FSH secretion. Changing GnRH pulse administration from a relatively high frequency (hourly) to a relatively low frequency (every 3 h) resulted in decreased LH but increased FSH secretion.

(Credit: Modified from Wildt L, et al: Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey, Endocrinology 109:376–385, 1981. Credit: Yen & Jaffe) [38].

responsiveness and continuous GnRH secretion may lead to near-complete desensitization of the receptors to GnRH. This desensitization to GnRH can be exploited for therapeutic treatment with GnRH agonists to turn off the HPG axis and induce a pseudo-menopause state. These medications have broad clinical applications and can be considered for the treatment of precocious puberty and endometriosis.

Additionally, different patterns of GnRH pulsatility differentially affect gonadotropes to induce transcription and production of LH or FSH. High-frequency pulses stimulate LH production, whereas low-frequency GnRH pulses favor FSH secretion [16]. In males, GnRH pulse frequency remains constant, whereas in females, pulse frequency varies throughout the menstrual cycle. LH pulse frequency, a surrogate for GnRH pulsatility, is slow in the luteal phase, speeding up during the follicular phase and culminates in a pre-ovulatory surge in conjunction with similarly timed GnRH surge secretion. Estrogen and progesterone released from the corpus luteum provide a negative feedback mechanism for GnRH post-ovulation.

Control/regulation of GnRH

Regulation of GnRH by neuronal inputs

KNDy neurons

Neurons in the arcuate nucleus also express several neurotransmitters including kisspeptin, neurokinin, and dynorphin which have been shown to regulate GnRH via a "pulse generator" and are essential for puberty and reproduction regulation [17]. These three neurotransmitters have lent their names to the KNDy neurons which produce these neurotransmitters [18].

Kisspeptin and its G-protein receptor GPR54 are responsible for normal pubertal development and loss of function mutations of the receptor cause hypogonadotropic hypogonadism [19,20]. This pubertal development is contingent upon careful synthesis and secretion of GnRH. Additionally, kisspeptin neurons express estrogen and progesterone receptors and are therefore sex-steroid dependent modulators of GnRH secretion. Neurokinin B stimulates kisspeptin and loss of function mutations in neurokinin B and its receptor TACR3 have been shown in humans to result in absent or delayed puberty, similar to kisspeptin [21]. Dynorphin, however, inhibits the release of GnRH [22].

Other neuropeptides

Several other neuropeptides impact GnRH either directly or through other intermediaries. Neuropeptide Y stimulates the appetite by way of insulin and leptin and has a potentiating effect on gonadotropins and indirect effect on GnRH. In the absence of estrogen, neuropeptide Y has been shown to inhibit gonadotropin secretion and is likely reflective of the body's nutritional status and acts as an indicator of the body's energy state in order to regulate reproductive function [23]. Glutamate stimulates GnRH secretion by activating nitric oxide as an intermediary which acts on cyclic GMP [24]. γ-aminobutyric acid (GABA) and noradrenaline appear to have dual roles on GnRH, both inhibiting and stimulating GnRH neurons. Serotonin, dopamine, and

prolactin have been shown to suppress GnRH pulsatility, although the pathways are not clearly understood.

Regulation of GnRH by gonadal feedback

In response to GnRH, gonadotropes produce both FSH and LH. In both males and females, FSH stimulates the production of gametes, while LH stimulates the steroid hormone production by the gonads. The androgens and estrogens produced suppress GnRH production via negative feedback at both the levels of the pituitary gonadotrophs and the arcuate nucleus. This is likely via the KNDy neuronal network, as GnRH releasing neurons do not have androgen or estrogen receptors themselves.

Regulation of GnRH by other regulating factors

Nutrition and stress also impact GnRH regulation and play a significant role in puberty and reproductive function. GnRH pulsatility is suppressed in energy-deprived periods such as stress and starvation. Insulin and leptin/ghrelin suppress the GnRH neurons via neuropeptide Y and increased cortisol from stress suppresses the KNDy neurons [25,26].

Clinical application of GnRH

Approximately 10%–30% of GnRH neurons are required to achieve normal physiologic function in the hypothalamus. However, the synchronized release of GnRH in a pulsatile fashion is necessary to achieve appropriate secretion of LH and FSH [27]. At the pituitary level, the expression of GnRH-Rs is dynamic and increases or decreases in response to feedback loops of FSH and LH, GnRH via an ultra-short feedback loop, and estradiol and progesterone [12].

GnRH has a half-life of 2–4 min, limiting its use as a therapeutic agent; pulsatile GnRH administration has been used to induce the development of follicles and induce ovulation; however, GnRH analogs have been developed with longer plasma half-lives in order to exert a desired clinical effect [28]. GnRH agonists bind to GnRH-Rs, mimicking the activity of native GnRH. Continuous administration of GnRH or a GnRH agonist (GnRH-a) leads to an initial stimulation of pituitary GnRH-R, known as the flare, followed by desensitization and downregulation of pituitary GnRH-R, resulting in suppression of gonadotrope secretion [28,29]. Pulsatile stimulation is necessary to avoid downregulation of the GnRH-R.

GnRH-a is utilized to both stimulate and suppress reproductive function according to the timing of the administration regimen. Pulsatile treatment mimics GnRH activity and maintains pituitary function, while continuous administration results in the suppression of pituitary gonadotropins following the initial flare effect [30]. GnRH-a can also be used as an alternative to hCG to trigger ovulation via induction of an LH surge in cycles utilizing GnRH-antagonists (GnRH-ant) for controlled ovarian stimulation, as the pituitary remains responsive to GnRH-a activity [28]. GnRH-antagonists are designed to shut down the pituitary-gonadal axis without the flare effect noted with GnRH or GnRH-a via competitive binding of GnRH to GnRH-Rs [31].

It has been demonstrated that GnRH/GnRH-R are located not only in the hypothalamus and pituitary, but also in the periphery, including in the ovary and endometrium [30]. Peripheral GnRH-R are associated with anti-proliferative activity and have been suggested as targets for GnRH-analog based therapies to treat ovarian tumors and other steroid dependent diseases [28].

GnRH in the ovary

GnRH/GnRH-Rs in the ovary play a role in control of follicular development and corpus luteum function. GnRH and GnRH-Rs were first shown to be expressed in the rat ovarian tissue and were confirmed to be highly expressed in the granulosa cells of pre-ovulatory follicles and in the granulosa luteal cells in the human ovary [32,33].

Expression is dynamic according to the stage of follicular development. GnRH-R in the granulosa cell layer of the graafian follicles and in granulosa luteal cells likely play a role in downregulation of the proliferation of human granulosa cells and apoptosis, regulating follicular development and atresia [34]. The GnRH/GnRH-R system is involved in autocrine and paracrine signaling in ovarian epithelial cell proliferation, as well as follicular development, atresia, luteinization, and luteolysis [28].

GnRH was also found to induce expression of genes involved in oocyte maturation and follicular rupture [35]. It has been reported that GnRH-a induce leuteolysis in the corpus luteum and stimulate matrix metalloproteinases, resulting in remodeling the extracellular matrix of the corpus luteum [35]. Functions of GnRH-R at the ovary remain incompletely defined and are areas of active research and future targets.

GnRH in the endometrium

GnRH/GnRH-Rs in the endometrium play a role in trophoblast invasion and embryo implantation. GnRH and GnRH-R are expressed in the human endometrium with dynamic changes associated with the menstrual cycle [28]. "Cross-talk" between the embryo and the endometrium occurs during implantation [30]. The endometrium expresses high levels of both GnRH and GnRH-R in the luteal phase and may play a role in trophoblast invasion during embryo implantation by regulating the proteolytic degradation of the extracellular matrix of the endometrial stroma and promoting motility of decidual endometrial stromal cells [36,37]. GnRH and GnRH-R are expressed in the cytotrophoblasts and syncytiotrophoblasts, with the highest levels of expression during the first trimester.

Endometrial GnRH may act in a paracrine fashion via activation of placental GnRH-Rs to contribute to regulation of hCG secretion [12]. In this way, it has been proposed that GnRH/GnRH-Rs are not only involved in the embryo/endometrium "cross-talk," but also in the hCG secretion to maintain progesterone production from the trophoblasts to support the pregnancy during the first trimester [30].

Inhibin

Structure

Inhibins are part of the transforming growth factor (TGF)-β superfamily [38]. They function to suppress the FSH secretion without affecting LH levels. Inhibins are glycoproteins produced by the granulosa and corpus luteal cells of the ovaries as well as the Sertoli cells of the testis [39]. The two main isoforms are inhibin A and inhibin B, which are disulfide-linked heterodimers [40,41]. They each share an identical α subunit and two different β subunits: β-A for inhibin A and β-B for inhibin B [38]. They are unique to the TGF-β family in that they act as antagonists and function primarily in an endocrine fashion [42].

Inhibins are synthesized as prohormones and intracellular cleavage of sulfhydryl-linked dimers releases mature C-terminal portions of the hormones to allow their biologic activity [43]. Mature inhibin ab dimers have molecular weights of 31–34 kDa [44].

Receptors

GnRH promotes both FSH and LH and does not differentiate between the two. Inhibins resolve this issue by specifically blocking pituitary FSH production. As such, they permit the separate modulation of LH and FSH. Inhibins have not been found to have signaling activity and function more as antagonists to activin-mediated signaling in the pituitary [42,43]. Activins are structurally related to inhibins and are potent stimulators of FSH production from gonadotropes [44]. Both, inhibin A and inhibin B, can form complexes with activin receptors to prevent activin's intracellular phosphorylation cascades, ultimately inhibiting the FSH production.

Betaglycan, a membrane bound proteoglycan, is an obligate and high affinity co-receptor for the α subunit of inhibin A. This complex of inhibin A and betaglycan then binds activin type II receptors (ActRII) A/B via inhibin's β subunit in pituitary gonadotrope cells to suppress FSH secretion [39,42,44]. Inhibin B is functional even in the absence of betaglycan and may have an unidentified co-receptor [42].

Production and inhibin expression in females

Inhibin is expressed in the ovaries, specifically in the granulosa cells of antral and pre-ovulatory follicles as well as in the granulosa luteal cells of the corpus luteum [39]. Levels of expression change throughout puberty and the menstrual cycle. Inhibin B is mainly produced by developing follicles while Inhibin A is predominantly produced by the corpus luteum. During the late phase of dominant follicle development, inhibin B concentrations are similar in follicular fluid and the serum whereas inhibin A is 4 times more concentrated in the follicular fluid [39,45].

Inhibin B serum levels rise in the early follicular phase, peaking after FSH, and slowly fall during the rest of the follicular phase. It peaks again after the LH surge,

and then rapidly decreases and remains low throughout the luteal phase. Inhibin A serum levels, on the other hand, are low in the early follicular phase, rise in the late follicular phase, and then peak at the mid luteal phase [46]. Inhibin A may also have a role in the negative feedback on FSH secretion during the transition to the luteal phase [39,47].

The finding of selective inhibin A & B expression in the menstrual cycle is further supported during pubertal development. Serum inhibin B levels rise several years before onset of puberty, suggesting high follicular activity without ovulation. Inhibin B further increases during Tanner stage 1 to 3 whereas inhibin A levels are not measurable until later in puberty after menarche and ovulation [39,48].

Inhibin has also been found to increase androgen production by theca cells. Due to increasing concentrations produced during follicular development, inhibins can have a paracrine effect on the adjacent theca cells and modulate LH-induced synthesis of androgens [39,49].

Aging women with regular menstrual cycles have increasingly high FSH levels, in the setting of normal estrogen and LH. This rise occurs several years prior to menopause and is concurrent with a decline of ovarian reserve. The discriminatory rise of FSH may be an effect of reduced levels of inhibins secondary to decreased numbers of ovarian follicles. It is also important to note that serum inhibin A and B are undetectable in postmenopausal women [39,50].

Production and expression in males

Sertoli cells produce inhibin A and B in male fetuses; however, in adult males, inhibin B is preferentially expressed and serum inhibin A levels should be undetectable. Prior to puberty, Sertoli cells produce both the α and the β-B subunits. After puberty, only the α subunit is expressed by Sertoli cells and production of the β-B subunit is taken over by maturating germ cells [39,51]. Inhibin B serum levels markedly rise during the pubertal transition from Tanner stage G1 to G2, which is concurrent with rising LH and testosterone.

Completion of spermatogenesis requires low FSH signaling and high LH signaling. This is accomplished by Sertoli cell inhibins suppressing FSH release from the pituitary. This is supported by the finding of infertile men with elevated serum FSH have an inverse correlation with inhibin B levels (Fig. 8.4) [39,52,53].

Summary

GnRH, produced and released by the hypothalamus is the key regulator in the HPG axis. Variations in pulsatility of GnRH production drive multiple physiologic functions including the production of gonadotropes FSH and LH and therefore play a pivotal role in reproduction. GnRH is regulated by many factors including an ultrashort feedback loop, KNDy neurons, and many other neurotransmitters in addition to diet, sleep, and stress patterns. Research has further demonstrated that GnRH also has effects in extra-pituitary tissues as well through the action of peripheral GnRH

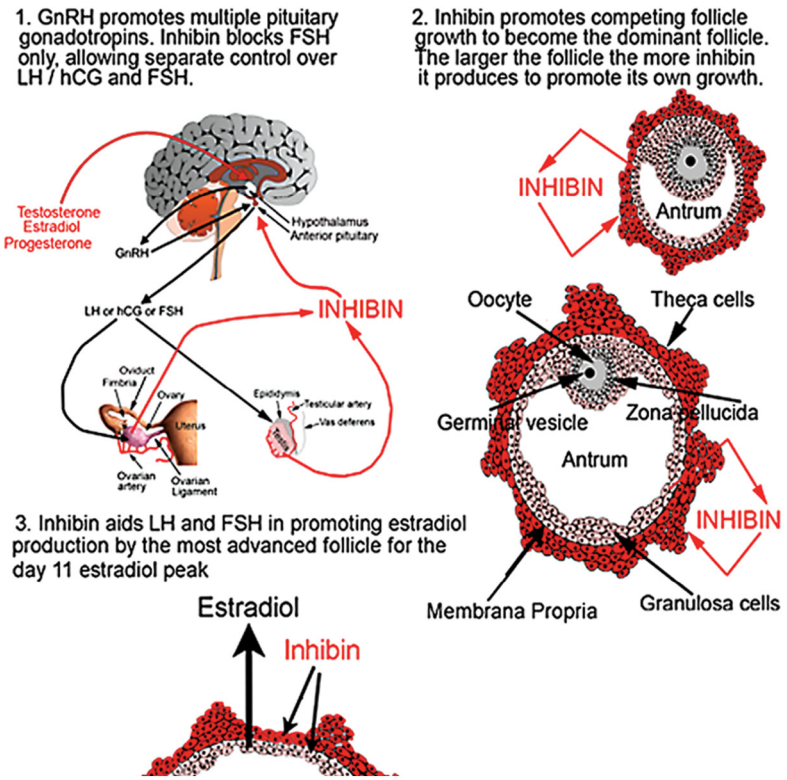

FIGURE 8.4

The principal function of inhibin in men and women.

receptors. New possibilities for novel treatment strategies, particularly in the locally expressed GnRH/GnRH-R system targeting the ovary and the endometrium.

Inhibin A and B are glycoproteins produced by the granulosa and corpus luteal cells of the ovaries as well as the Sertoli cells of the testis.

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