Hypothalamic-Pituitary-Testicular Axis

9

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Learning Objectives

- Structure of gonadotropin-releasing hormone (GnRH)
- Structure of GnRH receptor
- Synthesis and regulation of GnRH receptor
- GnRH-GnRH receptor interactions
- GnRH pulses
- Regulation of GnRH secretion
- Structure of gonadotropins
- Regulation of gonadotropin secretion

9.1 Introduction

Male reproduction, development, and maintenance of male sexual characteristics are principally governed by the hypothalamic-pituitary-testicular (HPT) axis. The axis is composed of the hypothalamic gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the gonadal steroids. GnRH is the central regulator of the male reproductive hormonal cascade. The HPT axis maintains a dynamic equilibrium of serum levels of reproductive hormones through a closed-loop feedback mechanism. A cause leads to an effect and that may be the end of a process. The cause and effect continue to modify each other. The effect may suppress the cause for attenuation of the effect. At other times, the effect may promote the cause for a still larger effect. The former is the negative

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feedback from the effect to the cause, and the latter is the positive feedback from the effect to the cause. Numerous hypothalamic, pituitary, and testicular factors tightly regulate the HPT axis and ensure its proper functioning.

9.2 Structure of Gonadotropin-Releasing Hormone (GnRH)

Localized within the hypothalamus is a group of 1,000 neurons which synthesize and secrete GnRH (Rance et al. 1994). GnRH neurons are fusiform in shape and generally bipolar. The axons project to the median eminence and infundibular stalk.

During the development of the embryo, GnRH-synthesizing neurons migrate from the olfactory area to the preoptic and arcuate nuclei of the hypothalamus. The migration of GnRH neurons is facilitated by a glycoprotein adhesion factor encoded by X-linked Kal-1 gene. Deficiency in the glycoprotein adhesion factor results in a condition referred to as Kallmann syndrome which is characterized by anosmia, GnRH deficiency, and hypogonadism (Rance et al. 1994).

In the mammalian hypothalamus, GnRH was first isolated as a decapeptide having pyro-Glu_1, His_2-Trp_3-Ser_4-$Tyrs_5$-$Glyg_6$-$Leur_7$-Arg_8-Pro_9-Gly_{10}-NH_2 structure (Millar et al. 2004). The gene for pre-pro-GnRH is located on the short arm of chromosome 8. GnRH is synthesized as a much larger 92-amino acid precursor peptide. The precursor peptide has four segments from N-terminal to the C-terminal: (1) signal peptide containing 23 amino acids, (2) GnRH decapeptide, (3) proteolytic site of 3 amino acids and (4) GnRH-associated peptide (GAP) containing 56 amino acids (Nikolics et al. 1985). The pre-pro-GnRH mRNA is encoded in four exons. Exon 1 contains the 5′ untranslated region of the gene; exon 2 contains the signal peptide GnRH and the N-terminus of GAP; exon 3 contains the central portion of GAP; and exon 4 contains the C-terminus of GAP and the 3′ untranslated region (see Fig. 9.1). The nucleotide sequences which

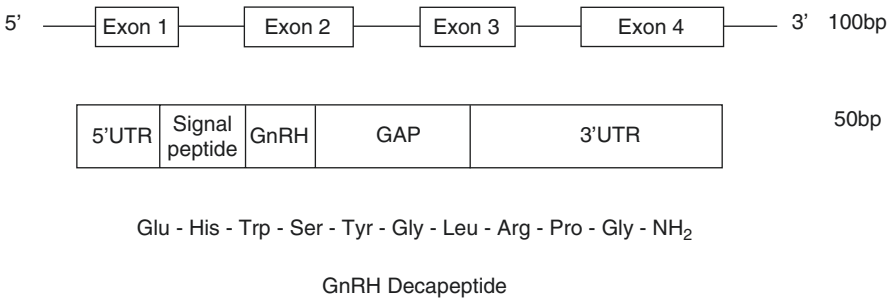

Fig. 9.1 Schematic diagram of the human gene for gonadotropin-releasing hormone (GnRH), the complimentary DNA (cDNA), and the gonadotropin-releasing hormone peptide; *bp* base pair, *UTR* untranslated region, *GAP* GnRH-associated peptide

encode the GnRH decapeptide are highly homologous. The precursor peptide is cleaved into decapeptide. The decapeptide is packed in storage granules. The storage granules are then transported to the median eminence (Milton et al. 1986).

9.2.1 Structure of GnRH Receptor

The specific receptor for GnRH is a typical seven-transmembrane domain G-protein receptor that belongs to type I rhodopsin-like receptor family. The seven hydrophobic transmembrane domains are connected via three extracellular loops (EL) and three intracellular loops (IL; see Fig. 9.2). The EL binds to GnRH, while the IL couples with G-protein and is involved in intracellular signal transmission. In humans, three GnRH receptors or receptor-like sequences have been identified: GnRH I, GnRH II, and GnRH II receptor-like homologue. The genes encoding the three receptors are located on chromosome 4, 1, and 14, respectively (Neill 2002). The gene encoding GnRH I and GnRH II is 40% identical to each other. The open reading frame of GnRH I and GnRH II gene consists of three exons spanning 18.9 kb. The three exons encode amino acids 1–174, 175–248, and 249–328, respectively. Between amino acids 174–175 in the transmembrane domain 4, intron 1 is located. Intron 2 is located between amino acids 248–249. The GnRH II receptor-like homologue gene is 40% and 100% identical to exon 2 and 3 of GnRH I and GnRH II gene, respectively. The GnRH II receptor-like homologue gene is encoded on the antisense DNA strand of the RM8 gene (Neill 2002).

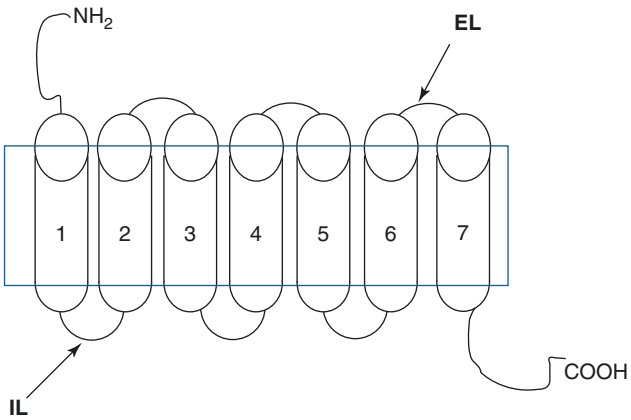

Fig. 9.2 The gonadotropin hormone (GnRH) receptor (*EL* extracellular loop, *IL* intracellular loop)

9.2.1.1 Synthesis and Regulation of GnRH Receptor

In rat pituitary cells, GnRH and protein kinase C (PKC) stimulate synthesis of GnRH receptor. However, GnRH and PKC utilize different mechanisms to stimulate GnRH receptor synthesis (Braden et al. 1991). Pulsatile secretion of GnRH every 30 min results in a maximum synthesis of GnRH receptor (Kaiser et al. 1997).

Estrogen stimulates GnRH receptor mRNA levels in rat pituitary tissue. Estrogen also increases the number of pituitary cells expressing GnRH receptor (Quiñones-Jenab et al. 1996). However, in adult rat pituitary cells, short-term administration of estradiol decreases GnRH receptor concentrations, while long-term administration increases the concentration of GnRH receptors (Emons et al. 1988). Progesterone inhibits the expression of GnRH receptor in the pituitary gland (Wu et al. 1994). Inhibin, a protein hormone, has no effect on the synthesis of GnRH receptors under basal conditions. However, inhibin blocks GnRH-stimulated synthesis of GnRH receptor (Braden et al. 1990). Activin A, another protein hormone, normally functions opposite to inhibin and stimulates synthesis of GnRH receptor in rat pituitary cell cultures. Inhibin does not block the stimulatory effect of activin A on GnRH receptor synthesis (Braden and Conn 1992).

9.2.2 GnRH-GnRH Receptor Interactions

When GnRH couples to its G-protein receptor, it can activate three G-proteins – stimulatory G-proteins: G_s and G_q, and inhibitory G-protein: G_i. When low concentrations of GnRH bind to its receptor, it activates Gs, which in turn activates adenyl cyclase (AC) that catalyzes the conversion of adenosine triphosphate (ATP) to 3′,5′-cyclic adenosine monophosphate (cAMP) and pyrophosphate (Krsmanovick et al. 2009). cAMP interacts with plasma membrane cyclic nucleotide-gated K^+ channels (CNG). The flow of ions across the plasma membrane causes a conformational change in the plasma membrane voltage-gated calcium channels (VGCC); it opens up the VGCC and allows calcium (Ca^{+2}) inflow from extracellular sites and raises the intracellular Ca^{+2} concentrations. When low concentrations of GnRH bind to its receptor, it also activates G_q. The activated G_q stimulates phospholipase C-β (PLC-β). PLC-β catalyzes the cleavage of phosphatidylnositol-4, 5-bisphosphate (PIP_2) into 1, 2-diacylglycerol (DAG) and inositol-1, 4, 5-trisphosphate (IP_3). The binding of IP_3 to calcium channels in endoplasmic reticulum (ER) turns on a downstream signaling cascade which results in the release of Ca^{2+} into the cytoplasm. The increase in Ca^{+2} by the above two pathways causes phosphorylation of certain downstream Ca^{+2}-dependent kinases that facilitates cytoplasmic GnRH release from the secretory organelles. At high GnRH concentrations, GnRH binding to its receptor activates G_i that inactivates cAMP and also interacts with G-protein-activated inwardly rectifying potassium channels (GIRK) that blocks plasma membrane VGCC, decreases intracellular Ca^{+2} concentrations, and therefore reduces GnRH production to baseline levels (see Fig. 9.3; Krsmanovick et al. 2009).

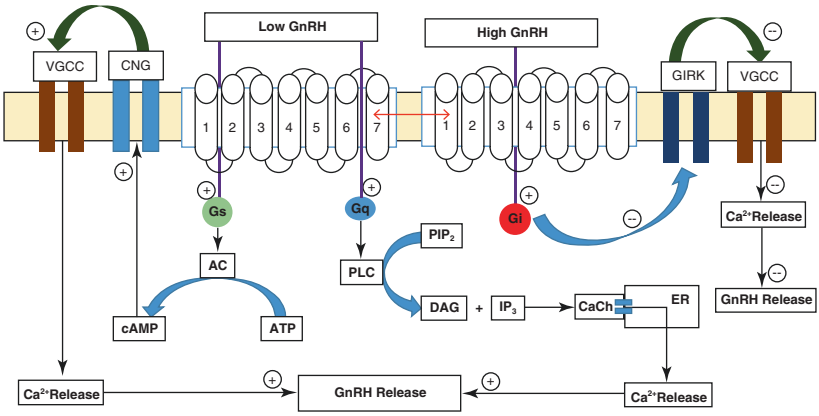

Fig. 9.3 Schematic diagram showing GnRH-GnRH receptor interactions (*GnRH* gonadotropin-releasing hormone, *Ca2+* calcium, *CaCh* calcium channel, *ER* endoplasmic reticulum, *DAG* 1, 2-diacylglycerol, *IP3* inositol-1, 4, 5-trisphosphate, *PIP2* phosphatidylnositol-4, 5-bisphosphate, *cAMP* cyclic adenosine monophosphate, *ATP* adenosine triphosphate, *AC* adenyl cyclase, *VGCC* voltage-gated calcium channels, *GIRK* G-protein-activated inwardly rectifying potassium channels, *PLC* phospholipase C, *CNG* cyclic nucleotide-gated K^+ channels, *Gs* and *Gq* stimulatory G-proteins, *Gi* inhibitory G-protein

9.2.3 GnRH Pulses

GnRH is secreted from the hypothalamic neurons in a pulsatile manner. The pulse-generating capacity of the GnRH neurons is intrinsic, i.e., even in the absence of any external influences, GnRH is released in pulses (Low 2011). The frequency of the GnRH pulses regulates the FSH to LH ratio from the pituitary. One pulse of GnRH every hour leads to a low ratio of FSH to LH secretion from the pituitary. One pulse of GnRH every 3 hr has an opposite effect on the FSH to LH ratio (Low 2011).

9.2.4 Regulation of GnRH Secretion

GnRH neurons are adjacent to other neuronal systems; thus, GnRH secretion can be modified by central nervous system inputs and neurotransmitters. Figure 9.4 shows different factors that regulate the HPT axis.

9.2.4.1 Gamma-Aminobutyric Acid

Gamma-aminobutyric acid (GABA) is synthesized from glutamate catalyzed by the enzyme glutamate decarboxylase (Soghomonian and Martin 1998). GABAergic neurons located in the anteroventral periventricular area (AVPV), lateral preoptic area, and suprachiasmatic nucleus show a direct action on GnRH neurons. All GnRH neurons express $GABA_A$ receptor, and majority of them express $GABA_B$ receptor (Herbison 2015). GABA is known to inhibit GnRH secretion. In female rats, GABA infusion into the preoptic area or intraperitoneal injection of the $GABA_A$ receptor agonist, muscimol, blocks the LH surge (Adler and Crowley 1986; Herbison

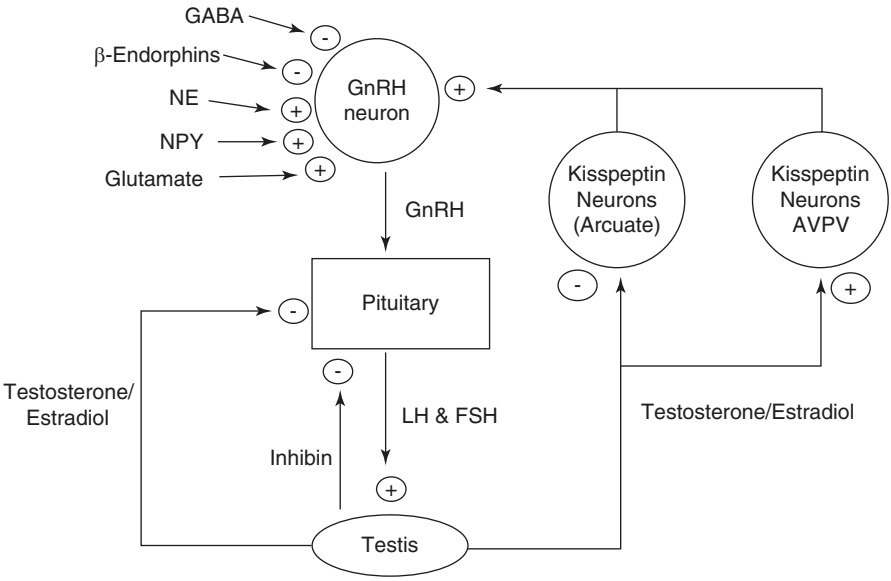

Fig. 9.4 Regulation of the hypothalamic-pituitary-testicular axis (*NE* norepinephrine, *NPY* neuropeptide Y, *GABA* gamma-aminobutyric acid, *GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *AVPV* anteroventral periventricular nucleus

and Dyer 1991). In female rats, intracerebroventricular injection of muscimol suppresses GnRH mRNA levels in the preoptic area (Bergen et al. 1991). Testosterone stimulates the expression of $GABA_A$ receptors (Zhang et al. 1999). Therefore, testosterone mediates its negative feedback effect on GnRH through GABA.

9.2.4.2 Glutamate

Excitatory amino acid, such as glutamate, is found in large concentrations in a variety of important hypothalamic nuclei, including the arcuate nucleus, supraoptic nucleus, suprachiasmatic nucleus, paraventricular nucleus, and preoptic area. Glutamate exerts its action through ionotropic and metabotropic receptors located on the GnRH neurons. Glutamate stimulates LH secretion by stimulating the release of GnRH (Brann 1995).

9.2.4.3 Norepinephrine

Norepinephrine (NE) is known to stimulate GnRH secretion. The stimulation is mediated by the β-1-adrenergic receptors (Segovia et al. 1996). Estrogen stimulates the release of NE from the mediobasal hypothalamus of female rats (Ohtsuka et al. 1989).

9.2.4.4 Neuropeptide Y

Neuropeptide Y (NPY) is a 36-amino acid peptide. NPY neurons are located in the arcuate nucleus and brainstem. NPY receptors are located on the GnRH neurons

(Herbison 2015). In male rats, NPY upregulates GnRH synthesis; the upregulation is mediated via Y1–NPY receptor (Li et al. 1994). NPY also acts at the level of pituitary, together with GnRH, to increase the LH secretion (Kalra et al. 1988).

9.2.4.5 Kisspeptin

Kisspeptin is a neuropeptide synthesized and secreted by the neurons in the hypothalamic arcuate, periventricular, and the AVPV nuclei (Dungan et al. 2006). Kisspeptin and GnRH-secreting neurons are located in a few common hypothalamic areas. The KISS1 gene encodes a 145-amino acid prepro-kisspeptin protein which is enzymatically cleaved into a 54-amino acid active protein known as kisspeptin-54. The kisspeptin protein is encoded by the KISS1 gene located on chromosome 1q32.1 in humans (see Fig. 9.5).

Kisspeptin binds to GPR54, a G-protein-coupled receptor. When kisspeptin binds to GPR54, it activates G-protein, Gq/G_{11}. Gq protein activates PLC. PLC in turn hydrolyzes PIP_2 into DAG and IP_3. IP_3 is released as a soluble structure into the cytosol. IP_3 then diffuses through the cytosol to bind to IP_3 receptors on Ca^{2+} channels in the ER. These channels open and only allow the passage of Ca^{2+}into the cytoplasm. DAG remains bound to the membrane. DAG activates the mitogen-activated protein kinase (MAPK) pathway (see Fig. 9.6; Pinilla et al. 2012).

Kisspeptin acts directly on the hypothalamic GnRH neurons where GPR54 mRNA has been identified (Irwig et al. 2004). Kisspeptin stimulates GnRH secretion and consequently LH and FSH secretion. The activation of GnRH is GPR54 receptor mediated as it is evident from mice lacking a functional GPR54 gene (Dungan et al. 2006).

The kisspeptin neurons in the arcuate and AVPV nuclei express androgen receptors and estrogen receptor-α (Smith et al. 2005). Kisspeptin neurons in the AVPV nucleus are different from those in the arcuate nucleus. In the AVPV, castration reduces both kisspeptin cell number and intracellular kisspeptin mRNA per cell (Smith et al. 2005). The stimulatory effect of estrogen on kisspeptin in the AVPV nucleus is responsible for the LH surge at mid-cycle in females. In female rats, the concentration of kisspeptin neurons in the AVPV nucleus is highest during the evening of proestrus (Smith et al. 2006). Most of the kisspeptin neurons in the AVPV nucleus co-express the Fos gene at the time of the LH surge. The product of

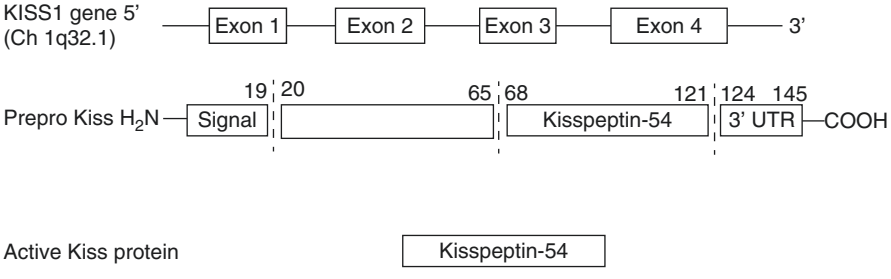

Fig. 9.5 Schematic diagram of kisspeptin gene and the corresponding protein

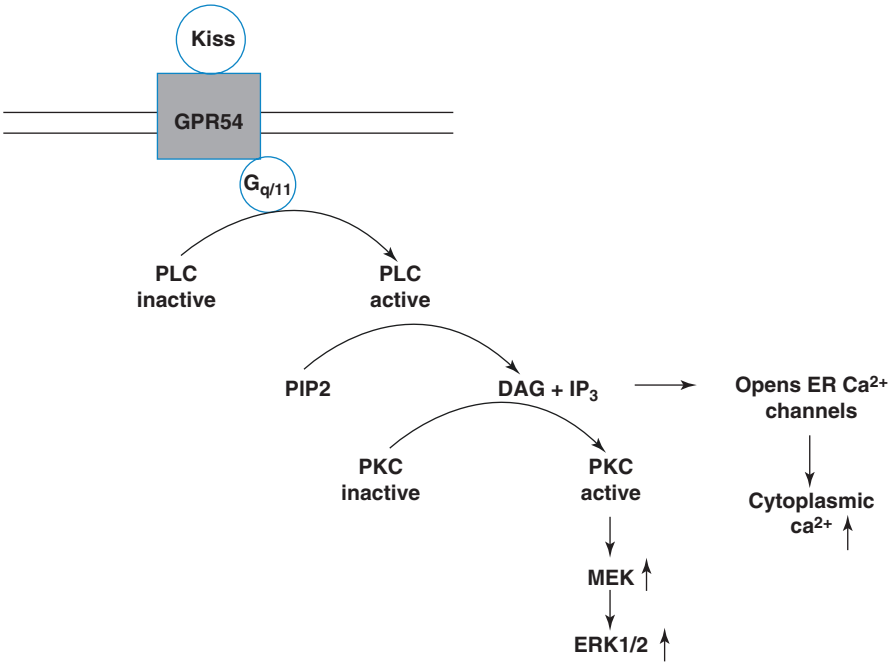

Fig. 9.6 The PLC-IP_3-DAG pathway activated by kisspeptin-GPR54 interaction (*KISS* kisspeptin, *GPR54* kisspeptin receptor, *PLC* phospholipase *C*, *PIP_2* phosphatidylinositol-4, 5-bisphosphate, *DAG* diacylglycerol, *IP_3* inositol-1, 4, 5-triphosphate, *ER* endoplasmic reticulum, *MEK* mitogen-activated protein kinase, *ERK* extracellular signal-regulated kinase

the Fos gene, a transcription factor, stimulates the proliferation of GnRH neurons and secretion of GnRH (Smith et al. 2006). In mice, histone of the kisspeptin gene locus is highly acetylated by estrogen which promotes the interactions among RNA polymerase and other transcriptional factors and thereby induce gene transcription.

Both estradiol and testosterone inhibit KISS1 gene expression in arcuate nucleus (see Fig. 9.7). Reduction of serum gonadal steroid levels by gonadectomy increases the number of kisspeptin neurons and also enhances the expression of KISS1–mRNA per cell (Smith et al. 2005). In contrast to the kisspeptin neurons in the AVPV nucleus, the expression of kisspeptin neurons in the arcuate nucleus is lowest during the evening of proestrus (Smith et al. 2006). Kisspeptin neurons in the arcuate nucleus do not co-express Fos gene at the time of the LH surge (Smith et al. 2006). In mice, histone of the kisspeptin gene locus in the arcuate nucleus show histone deacetylation in response to estrogen which condense the chromatin structure resulting in a subsequent inhibition in gene transcription.

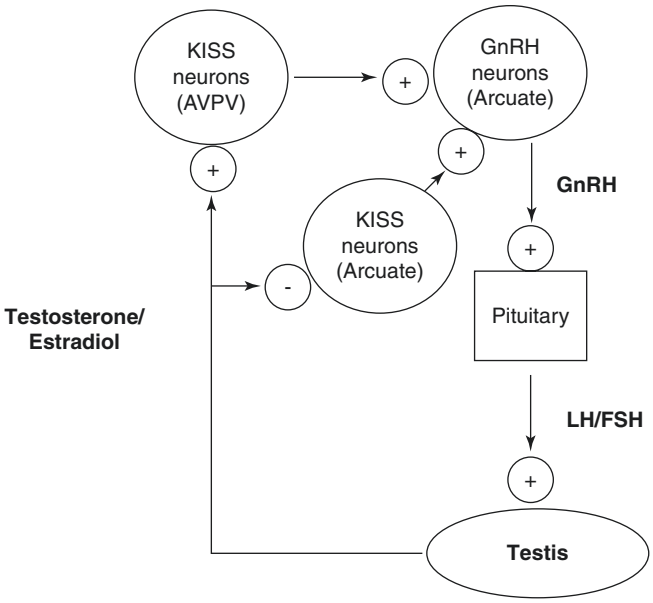

Fig. 9.7 Flow chart showing kisspeptin-mediated regulation of GnRH secretion (*AVPV* anteroventral periventricular, *GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone)

9.2.4.6 Endogenous Opioids

Endogenous opioid peptides are considered as important neuroendocrine regulators of GnRH secretion. Endogenous opioid peptides are located in cell bodies within the hypothalamus in the areas rich in GnRH neurons. Opioid receptors are located on the GnRH neurons (Low 2011). β-Endorphin from opioidergic system can decrease the secretion of GnRH, whereas NE neurons can stimulate its release (see Fig. 9.8; Ferin et al. 1984). Additionally, males addicted to heroin have been associated with central hypogonadism and decreased levels of gonadotropins and gonadal steroids (Katz 2005). In rats, treatment with morphine, an opiate, significantly decreases basal and human chorionic gonadotropin (hCG)-stimulated testosterone levels and testicular interstitial fluid volume (Adams et al. 1993). Intratesticular opiates inhibit Sertoli cell growth and androgen-binding protein secretion (Gerendai et al. 1986).

9.2.4.7 Gonadal Steroids

Gonadal steroids – testosterone and estradiol – exert a negative feedback effect on the HPT axis (see Fig. 9.9). The GnRH neurons lack androgen or estrogen receptors (Low 2011). Therefore, the gonadal steroids regulate GnRH by acting on the afferent neurons synapsing with GnRH neurons. At the hypothalamic level, all the

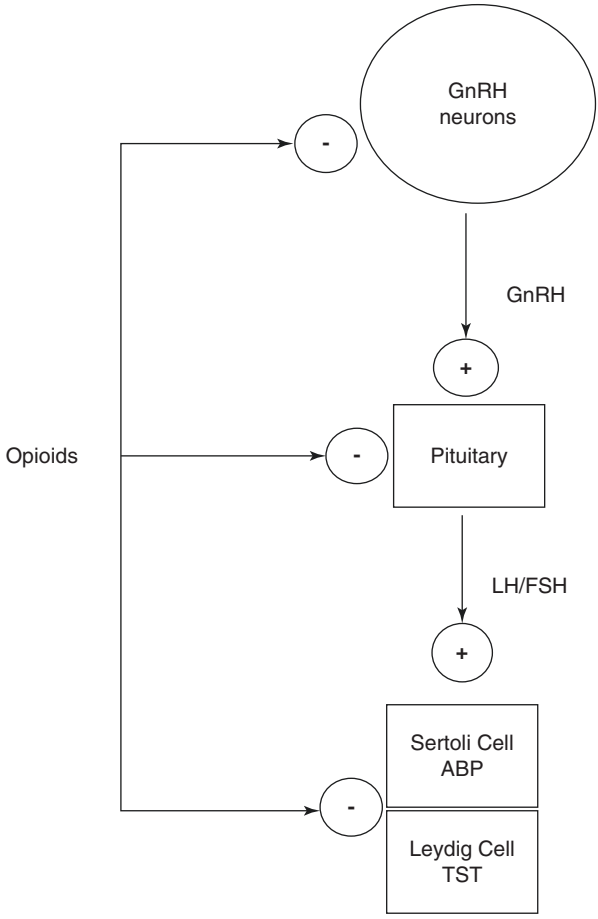

Fig. 9.8 Flow chart showing interaction between opioids and the hypothalamic-pituitary-testicular axis. *GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *ABP* androgen-binding protein, *TST* testosterone

gonadal steroids reduce the frequency of GnRH pulse probably by altering neurotransmitter inputs from the afferent neurons impinging on GnRH neurons. Gonadal steroids also regulate KISS1 mRNA expression which further regulates GnRH secretion as discussed in the previous section.

9.3 Structure of Gonadotropins

The pituitary gland or hypophysis is found at the base of the brain below the hypothalamus. The infundibulum or pituitary stalk which carries both axons and blood vessels, serves as a link between the hypothalamus and the pituitary gland. The pituitary gland can be divided into two functionally and embryologically distinct parts/lobes – the anterior pituitary or adenohypophysis and the posterior pituitary or

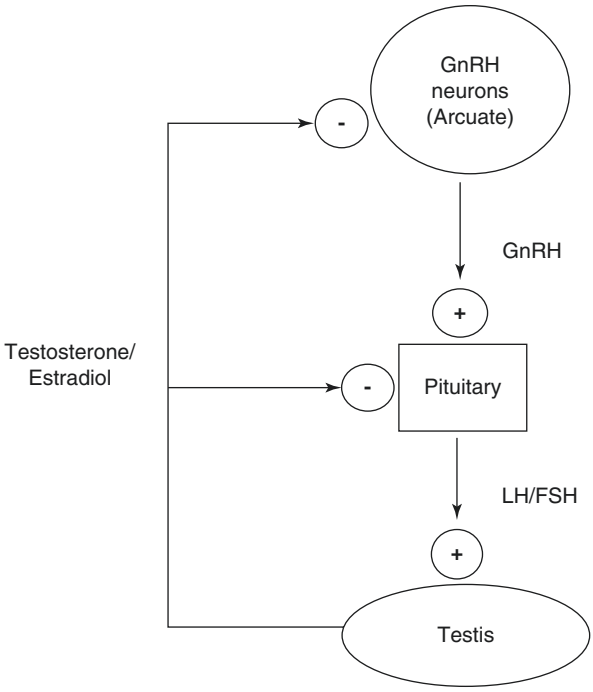

Fig. 9.9 Flow chart showing regulation of hypothalamic-pituitary-testicular axis by gonadal steroids; *GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone

neurohypophysis. The anterior pituitary cells which synthesize and secrete gonadotropins are called gonadotrophs. Gonadotrophs secreting LH and FSH constitute 7–14% of the cells in the pituitary (Manjithaya and Dighe 2006). Pituitary cells also synthesize adrenocorticotropic hormone, growth hormone, thyroid-stimulating hormone (TSH), and prolactin. A specialized capillary network, the pituitary portal system, located between the median eminence of the hypothalamus and the pituitary, transports neuropeptides between these two structures. The portal circulatory system delivers neuropeptides from the hypothalamus to pituitary. Through retrograde blood flow, the circulatory system allows communication between anterior pituitary and the hypothalamus. The communication is also possible between anterior and posterior parts of pituitary gland (Lechan and Toni 2000).

GnRH is transported directly to the pituitary gland via the portal circulation where it binds with its specific cell surface receptors and stimulates the anterior pituitary gland to release two glycoproteins – LH and FSH in a pulsatile fashion. LH and FSH are protein dimers consisting of two glycosylated polypeptide chains-alpha (α) and beta (β), which are non-covalently linked together. The α-subunit of the glycoprotein hormones LH, FSH, TSH, and hCG is identical. However, the β-subunit structure is unique for each hormone and determines specificity for their different receptors. In humans, the genes encoding the common α-subunit are located on chromosome 6, while the β-subunits of FSH and LH are located on

chromosome 11 and 19, respectively. Circulating free-α and free-β subunits are not recognized by gonadotropin receptors and are, therefore, are not biologically active (Bousfield et al. 2006).

Posttranslational processing of the gonadotropins involves glycosylation or addition of carbohydrate side chains by microsomal enzymes in the ER (Catt and Pierce 1986). The common α-subunit has two glycosylation sites at amino acid 52 and 78, while the glycosylation sites on their β-subunits are 7, 24, and 30, respectively. The oligosaccharide structures in both LH and FSH have a central mannose core, bound to an asparagine (Asn) residue. In LH, the Asn residue is bound through two residues of N-acetylglucosamine and a terminal sulfate while in FSH through a sialic acid residue. The terminal N-acetylglucosamine sulfate in LH is quickly removed from circulation by liver receptors that recognize the terminal sulfate. In contrast, the terminal sialic acid residue in FSH protects it from being captured by liver receptors. As a result the half-lives of LH and FSH in peripheral circulation vary widely, i.e., approximately 20 and 180 min, respectively (Bousfield et al. 2006).

The net surface charge on LH and FSH also determines their biological activity and half-lives. The more basic form of LH and FSH results in a greater in vitro potency but a shorter half-life in circulation, while reverse effects are observed for the less basic forms (Stanton et al. 1996).

The release of gonadotropins in response to GnRH has two unique properties. Continuous administration of GnRH results in an initial rise in the levels of LH and FSH. However, after several days of exposure to GnRH, secretion of LH and FSH gradually diminishes. Continuous exposure to GnRH for a prolonged period results in diminished GnRH binding or downregulation of its receptors. The downregulation is a reversible process and is accompanied with decoupling of receptor from G-protein and internalization of the receptor from the surface and its degradation in lysosomes (Clayton and Catt 1981). Downregulation and diminished secretion of LH and FSH can also be induced by the administration of long-acting synthetic agonist analogues of GnRH. The GnRH agonists can reduce LH and FSH levels and abolish gonadal function. Pulsatile administration of GnRH at a physiological frequency of 60 min to 4 h increases GnRH receptor binding on the gonadotroph and consequently increases LH and FSH levels by a process called upregulation (Bousfield et al. 2006).

The differential release of FSH and LH is also related to GnRH pulse frequency (Belchetz et al. 1978). One pulse of GnRH every hour may lead to greater release of LH. A slower frequency, i.e., one pulse of GnRH every 3 h, may stimulate FSH secretion with low secretion of LH. It has been observed that at the onset of puberty when GnRH pulse generator begins to operate, the ratio of FSH to LH is greater than the adults (Burr et al. 1970).

When FSH binds to its receptors located on the plasma membrane of Sertoli cells, it activates the cAMP-dependent protein kinase A (PKA) pathway. PKA phosphorylates cAMP-dependent response element-binding proteins. Through this pathway, specific Sertoli cell genes for various proteins such as inhibin, activins,

anti-Mullerian hormone, estrogens, androgen-binding proteins, transferrin, ceruloplasmin, and a variety of proteases including plasminogen activator, cathepsin L, and several metalloproteases are activated. Leydig cells have specific membrane receptors for LH. Binding of LH to its receptors on Leydig cells activates a cAMP-dependent PKA pathway which activates enzymes required for testicular steroidogenesis (Lo and Lamb 2004).

9.3.1 Regulation of Gonadotropin Secretion

The secretion of gonadotropin is regulated by GnRH and testicular steroids. The regulation of gonadotropin secretion by GnRH has been discussed in the previous section. We shall now describe the role of testicular steroids in regulating gonadotropin secretion.

9.3.1.1 Role of Androgens

The production of gonadotropins is negatively controlled through an inhibitory action of androgens both at the central nervous system and the pituitary level. Androgens exert a negative feedback effect on gonadotropin release from the pituitary. The negative feedback effect of androgen decreases responsiveness of the pituitary to GnRH, thereby resulting in decrease in LH pulse amplitude and fall in plasma LH levels (Sheckter et al. 1989). Testosterone also exerts its negative feedback effect on GnRH release by stimulating release of opioid peptides from hypothalamic neurons. Opioids inhibit pulsatile GnRH secretion from the hypothalamus and consequently reduce LH levels (Veldhuis et al. 1984). Testosterone is reduced to non-aromatizable dihydrotestosterone (DHT) in the brain and peripheral tissues. DHT does not have any effect at the pituitary level but exerts a negative feedback effect on LH secretion at the hypothalamic level (Cailleux-Bounacer et al. 2009). Administration of DHT to young men has been shown to significantly decrease immunoactive FSH levels without any change in bioactive FSH levels (Urban et al. 1991).

9.3.1.2 Role of Estrogens

Testosterone aromatizes to estradiol in the brain and peripheral tissues. Estrogens inhibit pituitary LH secretion by decreasing hypothalamic GnRH pulse frequency and also the pituitary responsiveness to GnRH. All estrogen-mediated effects can be reversed by administration of anti-estrogens or aromatase inhibitors (Hayes et al. 2000). It is the level of circulating estrogen, but not of testosterone, that regulates the LH levels in males. In hypothyroid men, the testosterone levels decrease, but estrogen and LH levels do not change suggesting that estradiol is of primary importance in the regulation of LH secretion in males (Kumar et al. 2007). In hyperthyroid men, a hyperestrogenemic condition exerts a positive feedback effect on LH secretion instead of a negative feedback (Kumar et al. 2012). Injection of 17β-estradiol to normal adult males results in an initial fall in FSH

and LH followed by an increase in LH alone (Kulin and Reiter 1976). Maintenance of serum estradiol concentration similar to that present in women at mid-cycle, for a period of 96–122 h, leads to a surge of LH in both intact and castrated men (Barbarino et al. 1983). However, the magnitude of LH surge was not as huge as found in females which is probably because of high testosterone levels seen in men (Barbarino et al. 1983, Kumar et al. 2012). The exact cause for the switch from a negative feedback effect to a positive feedback effect of estrogen on LH levels is not known in males. However, the mechanism of positive feedback effect of estradiol in males can be the same as that seen in mid-cycle women. Estradiol levels of 200–400 pg/ml persisting for at least 36 h are adequate to generate LH surge in women at mid-cycle (Karsch et al. 1973). The role of kisspeptin in the AVPV nucleus during the LH surge has already been discussed previously in this chapter. Within the pituitary, estradiol increases GnRH receptor expression, resulting in increased pituitary sensitivity to GnRH pulses (Nakai et al. 1978). Hypothalamic GnRH secretion is also increased at the time of LH surge, as directly measured in sheep and rats; however, this increase is not essential for production of LH surge (Ehlers and Halvorson 2013). In lower animals such as rodents, the behavior of HPT axis is rigidly dimorphic and cannot be altered by changing serum hormonal levels. It can be concluded that in primates and humans, steroid-mediated feedback mechanism is epigenetically and endocrinologically determined.

Estrogen administration has been shown to delay the clearance of FSH from circulation by decreasing the half-life of FSH, but it does not change FSH pulse frequency.

9.3.1.3 Role of Inhibin and Activin

FSH secretion is also partly regulated by inhibin and activin, which belong to the tumor growth factor-β (TGFβ) protein superfamily. Inhibin, a nonsteroidal protein hormone, is secreted by Sertoli cells under the stimulation of FSH. The protein is composed of two dissimilar subunits, an α-subunit with molecular weight 18,000 Da and a β-subunit with molecular weight 14,000 Da. Both subunits are cross-linked by disulfide bridges. Two forms of inhibin have been identified – inhibin A and inhibin B. Inhibin B is the predominant form in primates including humans (Childs 2006). Both forms share the same α-subunit but have differences in the peptide sequence of the β-subunit. Inhibin has been shown to suppress FSH secretion preferentially (Mason et al. 1985). When castrated male ram is infused with physiological concentration of inhibin, it results in suppression of FSH levels to that in normal male ram. Moreover, when inhibin is administered in these castrated males treated with GnRH, it still suppresses FSH levels. This suggests that inhibin acts exclusively on the pituitary gland (Tilbrook et al. 1993). Inhibin also exerts its negative effect on pituitary gland by antagonizing the effect of activin. Activin is composed of two β-subunits of inhibin and stimulates FSH synthesis and secretion. Activins are produced by anterior pituitary gonadotrophs and testicular Sertoli cells. Pituitary activins behave as autocrine modulator of basal FSH secretion as suggested in experiments using anti-activin antibody. Administration of

anti-activin antibody inhibits pituitary FSH β-subunit levels without affecting α-subunit levels or LH levels (Corrigan et al. 1991).

Key Questions

- Describe the segments of the precursor peptide of GnRH.
- Describe the GnRH receptor-mediated signaling cascade downstream of GnRH receptor binding.
- How do kisspeptin and opioids mediate regulation of GnRH secretion?
- Describe the structure of LH and FSH.
- Discuss the role of testicular steroids and protein hormones in regulation of gonadotropin secretion.

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