

## Kisspeptin: Key Regulator of Hypothalamic–Pituitary–Gonadal Axis

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### ABSTRACT

**BACKGROUND AND OBJECTIVE:** Gonadotropin-Releasing Hormone (GnRH) neurons of hypothalamus are final output of brain for regulation of puberty onset and hypothalamic pituitary gonadal (HPG) axis functions in mammals. However, *the mechanisms responsible* for release of GnRH neurons are unknown. A number of various factors including neurotransmitters, neuropeptides or different signals have been identified to be involved in the regulation of the secretion of GnRH neurons. Neuronal set of kisspeptin have been recognized recently as critical upstream regulators of GnRH neurons. Given the importance of this issue, in this study a review of various studies and sources about biosynthesis, neuroanatomy, signaling, function and dysfunction of kisspeptin was performed.

**METHODS:** In this review study, new evidence in relation to role of kisspeptin neuropeptide in the reproductive system were investigated by using various databases including pubmed, sciencedirect, nature, springer, wiley, scopus and key words such as kisspeptin, gonads, hypothalamus, GnRH and reproduction were used.

**FINDINGS:** From 145 gained articles, 63 articles were reviewed. Kisspeptin neuropeptide signaling in hypothalamus is required for initiation of puberty and mammalian reproductive function. Kisspeptin neurons stimulate GnRH release and act as central integrator of external and internal signals. Neurones kisspeptin are sensitive to sex steroids, metabolic cues estrogen like compounds.

**CONCLUSION:** Kisspeptin neurons play a vital role in the maturation and function of the HPG axis, including the sexual differentiation of the brain, the timing of puberty, the regulation of gonadotropin secretion and the control of fertility by hormonal and environmental cues.

**KEY WORDS:** *Kisspeptin, Gonads, Hypothalamus, Gonadotropin-Releasing Hormone.*

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## Introduction

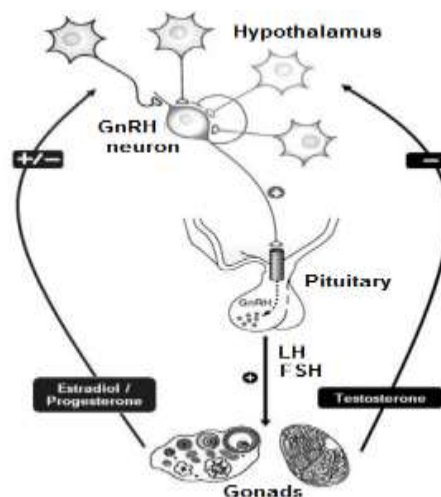
Reproductive ability is essential for the survival of all species, including mammals. Adjusting the reproductive system in mammals, including the setting of puberty, the menstrual cycle, gametes, fertility, and ultimately menopause, all originate from the brain (1). The brain regulates reproductive function through the hypothalamic-pituitary-gonadal axis (HPG) axis. A series of neurons located in the hypothalamus called Gonadotropin-Releasing Hormone (GnRH) neurons receive various messages from other neurological pathways. These messages lead to various physiological conditions, including the feedback of steroids, stress, nutrition, and other metabolic states, energy, and circadian rhythms to the GnRH neurones, and ultimately play an essential role in regulating GnRH leakage (2).

In most mammalian species, GnRH neurons are scattered through the division of the preoptic area (POA) of the hypothalamus. The GnRH neurons send their axons to the mid-prominence, which is the ultimate brain outlet in the control of the reproductive axis (4, 3). In the mid-prominence, GnRH is pumped into the blood vessels of the pituitary bubble and transmitted through the bloodstream to the anterior pituitary (Fig. 1). In the anterior pituitary, neurohormone GnRH affects gonadotrophic cells and with the help of other hormones, including sex steroids, regulates the secretion of gonadotropins, the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) (5).

Gonadotropins have reached the gonads (ovaries and testicles) through the bloodstream, where they regulate the growth of follicles and ovulation in females, and spermatogenesis and sperm maturation in males. Gonadotropins also stimulate the production and secretion of sex steroids, estradiol and progesterone in females and testosterone in males (Fig. 1) (6,1). The regulation of GnRH secretion is the main mechanism in which the body is able to control its reproductive status during the process of puberty and reproductive capacity after it (7).

Up to now, a number of regulators have been identified that affect the level of GnRH secretion, including sex hormones, neurotransmitter mediators, neuropeptides, stress, metabolic and environmental messages that are able to change the function of the HPG axis (9, 8). Several neurotransmitter and neuropeptide-afferent populations (Figure 1), including GABA, Dopamine, Glutamate,  $\beta$ -Endorphin,

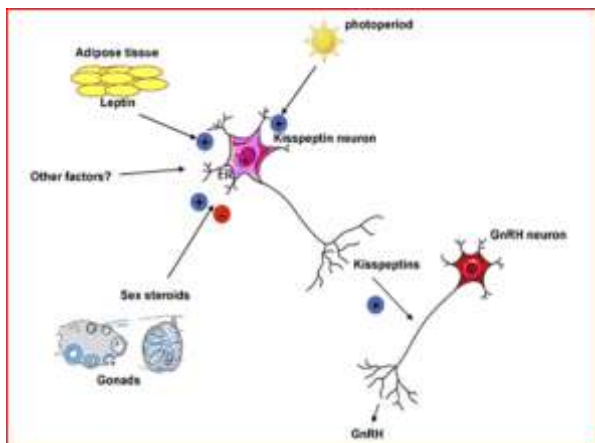
Dinorphine, Gallanthine, etc., have been identified through synaptic relationships with GnRH neurons and their ability to regulate gene transcription, protein production, and ultimately GnRH secretion. But one of the most important neuronal networks identified early in the century that has a key role in regulating reproductive activity is the kisspeptin system (10-13).



**Figure 1. The hypothalamus-pituitary-gland axis in mammals. The GnRH neuropeptide affects the anterior pituitary to stimulate the secretion of gonadotropins (LH and FSH). Gonadotropins reach the gonads through the bloodstream (ovaries and testicles) and cause the secretion of sex hormones. At the level of the hypothalamus, estrogen exerts its effects through negative and positive feedback and testosterone with negative feedback to regulate GnRH secretion. Various neurons also participate in the administration of steroidal messages and other environmental and central conditions to the GnRH neurons (6).**

The progress of the past decade in our understanding of the mechanisms for controlling the secretion of GnRH is due to the discovery of kisspeptin and its vital role in reproduction (6). kisspeptin neural network is identified by unifying the central and peripheral messages including photoperiodic signals, leptin hormone secreted from adipose tissue, sex hormones, and other factors including neural mediators (Fig. 2) as a key upstream regulator for activating GnRH neurons and its products (17-14). Presently, the kisspeptin system is considered to guide the feedback effects of sex steroids into GnRH neurons as a key regulator of GnRH secretion in puberty and adulthood (17, 16). One of the main evidence for this role of kisspeptin in the HPG axis is the high

expression of estradiol, progesterone, and testosterone receptor in the kisspeptin neurons (19, 18). Considering the importance of the subject, in this study, using different studies and various information sources, the effect of kisspeptin on the reproductive axis was investigated.



**Figure 2. The role of the kisspeptin system in integrating central and peripheral messages in the reproductive axis (14)**

## Methods

In this review, we use the pubmed, sciencedirect, nature, springer, wiley, scopus and key words of kisspeptin, gonads, hypothalamus, GnRH, reproduction, endocrine intervening compounds, new evidence in relation to construction, phylogeny, neuroanatomy, sexual dysfunction, and the effect of sex steroids on kisspeptin neurons, messenger and kisspeptin receptor, the role of kisspeptin in the reproductive axis, and the effect of endocrine intervening compounds on kisspeptin system were investigated.

## Results

Of the 145 papers obtained from various databases, 63 related articles were reviewed, and other articles that did not relate to the subject of this study were excluded from the study. Our study showed that kisspeptin acts as a key actor in regulating the onset of puberty and natural performance of reproductive axis.

**Construction and phylogeny of kisspeptin:** The Kiss1 Gene, originally, encodes the leading peptide of Prokisspeptin with 145 amino acids. This peptide is converted to the active form of a kisspeptin that has 52 to 54 amino acids (Kp52, Kp53, and Kp54) after proteolytic activity (20). The subsequent proteolysis of

caseipetin produces shorter peptides (Kp14, Kp13, Kp10), all having the same amino acid region at the carboxyl end. Shortened kisspeptin, similar to the active peptides Kp52, Kp53 and Kp54, are capable of activating GPR54 (22, 21). The amino acid sequencing of kisspeptin has been well preserved among mammals. For example, goat kisspeptin sequence indicates 98%, 91%, and 77% of the same amino acid sequences, especially in 10 amino acid of carboxylic end, respectively, with sheep, bovine and swine (24, 23). However, it should be noted that full-length kisspeptin in either rat (Kp52), sheep (Kp53) or human species (Kp54) appear to have more potential bioavailability than Kp10 (25, 24).

**Neuroanatomy of kisspeptin neurons:** The distribution of kisspeptin neurons in the hypothalamus differs between different species (26, 23). The results of In situ hybridization studies and immunohistochemistry in rodents show the accumulation of kisspeptin peptides in two separate and distinct nuclei of the hypothalamus.

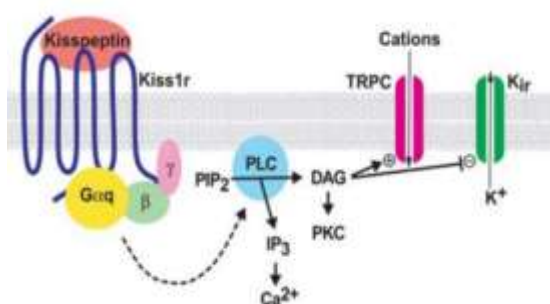
One of these centers is the presence of kisspeptin neurons in the arc nucleus (Arcuate Nucleus=ARC) and the other in the part of the POA in the anteroventral periventricular nucleus = AVPV (23, 27-29). Human, avian monkeys and sheep show a higher ratio of kisspeptin peptic neurons in ARP nucleus than AVPV (30-32). In rodents and monkeys, the expression of kisspeptin in ARC and AVPV nuclei increases in pre-puberty, which is consistent with the messenger of kisspeptin as the initiator of puberty (28,32,33). In adult female rodents, most neurons of kisspeptin are found in the POA at the core of AVPV, and the axonal terminals of these neurons have synapses with the cellular body of the GnRH neurons (23, 28). These synaptic connections of AVPV kisspeptin neurons are likely to play a role in modulating the GnRH/LH peak before ovulation in females (23,34). But neuronal terminals of kisspeptin from ARC origin have synapse with axon of GnRH neurons in the mid-prominance of the monkeys and may play a role in modulating the GnRH pulse secretion (23,35).

**Sexual dimorphism and the effect of sex steroids on kisspeptin neurons:** In some species, there are anatomical differences in neuronal density and in the size of some hypothalamic nucleus of males and females (36). In rodents, AVPV is a bifocal gender and has a greater number of neurons and larger nucleus in females than males. This sexual dimorphism with the

administration of neonatal testosterone, which is aromatized to estrogen in the brain, causes maturation of the AVPV region (28,31). Female newborn mice treated with androgen or estradiol develop a smaller number of neurons in AVPV, but castration of the male neonate rats increase the number of kisspeptin neurons in AVPV (35,36).

Similarly, in male transgenic mice lacking the GPR54 receptor similar to that of female mice, it has an AVPV region with more kisspeptin neurons than wild type males (37). These findings suggest that kisspeptin messenger for AVPV needs testosterone production during the neonatal period. Unlike AVPV, the ARC nucleus does not show a gender dimorphism in the density or distribution of kisspeptin neurons in adult rodents (35, 37).

**Signaling and kisspeptin receptor:** The kisspeptin receptor, Kiss1r (GPR54), is a cell-surface receptor that belongs to the family of receptors with G-protein coupled receptors. kisspeptin binding to Kiss1r leads to the activation of beta-phospholipase C (PLC $\beta$ ), followed by the hydrolysis of phosphatidylinositol 4 and 5 bisphosphate (PIP<sub>2</sub>) and the production of intracellular secondary transducers namely, inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG), which results in the release of significant amounts of intracellular calcium and activation of protein kinase C (PKC), respectively. kisspeptin is thought to eventually trigger the release of GnRH in two ways. One of the pathways is the activating the transient receptor potential cation channels (TRPC) and the entry of cations into the neurons and the other by closing Kir type potassium channels and preventing the release of potassium ions from the neurons, possibly due to DAG and / Or Ca<sup>2+</sup> + (Fig. 3) (38, 21, 20).



**Figure 3. The mechanism of GnRH neuronal stimulation by binding the kisspeptin to the Kiss1r receptor (21).**

**The role of kisspeptin in the reproductive axis:** The essential role of kisspeptin in the regulation of the

function of reproductive axis has been demonstrated by investigating the mutations in the signaling pathways of rodents and humans. Transgenic mice with the absence of the kisspeptin receptor gene (kiss1r) or the lack of the Kiss1 gene do not progress the development of sexual maturity and are infertile in both males and females (37-41). These mice have defects in the development of gonads and malformations in gametes. Male rats also produce less sperm count, and rats do not show normal estrous cycle, and ovarian defects and corpus luteum deficiency are observed in their ovaries (39, 37 and 23). In addition, in these mice, low levels of gonadotropins and sex steroids have also been observed in the bloodstream (37,40,41). In humans, mutations in GPR54/Kiss1r similarly lead to hypogonadotropic hypogonadism(23,40,40).

The presence of a missense mutation (a point mutation in which a change in a nucleotide can cause a change in the formation of another amino acid instead of the main amino acid in the polypeptide chain), in the kisspeptin precursor protein in a Brazilian boy with precocious central puberty has been identified (42). These reproductive defects may be directly related to the action of kisspeptin in the hypothalamus. In humans and some species, central or peripheral injection of kisspeptin, stimulates the secretion of gonadotropins. In concordance with this finding, most GnRH neurons express kisspeptin receptors (24, 23). GnRH secretion is demonstrated by mediation of kisspeptin in *Macaca mulatta* and ewes, and by inhibiting kisspeptin responses in rodents with the administration of GnRH antagonists (43). In addition, GPR54/Kiss1r disrupted genetic mice can not secrete GnRH after stimulating casein peptide (44).

As previously mentioned, sex steroids provide feedbacks and allow the gonads to affect the hypothalamus to regulate the secretion of GnRH (Fig. 1). Because GnRH neurons do not express the androgen or estrogen receptors of alpha, sexual steroids indirectly do this (46, 45). kisspeptin neurons are now thought to mediate the effect of sex steroids on GnRH neurons.

Most kisspeptin neurons (90%) express alpha-type estrogen receptors, androgen receptors (65%) and progesterone receptors (86%), that is consistent with their role as mediators of steroid feedback in the reproductive axis (47,24,23 ). In rodent, sex steroids differentially regulate the kisspeptin in ARC and AVPV nuclei. These changes are reversed by either

testosterone or estradiol. Studies in other species have confirmed the effects of sex steroids on the expression of kisspeptin (47, 23,1). In sheep, kisspeptin neurons of the ARC nucleus mediates both negative progesterone feedback on GnRH secretion during luteal phase and the positive feedback action of estradiol during ovulation (24, 23). The mechanism by which sexual steroids differentially express the kisspeptin in the ARC and AVPV nuclei are not well understood.

In rodents, estradiol mediates its feedbacks through both ERE-dependent and ERE-independent messaging. This study examines the effects of estradiol on Kiss1 expression in mutant mice with ER $\alpha$  molecules that are not capable of binding to the ERE sequence. These studies have shown that stimulating the expression of Kiss1 by estradiol in AVPV requires an ERE-dependent pathway. Conversely, inhibiting the expression of Kiss1 by estradiol in the ARC nucleus requires an ERE-independent pathway (48, 24). In addition, insulin-like growth factor-1 in the presence of estradiol increases the expression of Kiss1 in female mice at pre-pubertal age in AVPV, but does not increase ARC nucleus.

These changes in the expression of kisspeptin in the ARC nucleus are in line with the effects of negative feedback regulation of sex steroids on the release of GnRH and in AVPV, consistent with the response to LH prior to ovulation (23).

**Endocrine disrupting compounds and kisspeptin system:** Endocrine disrupting compounds (EDCs) are a heterogeneous group of natural compounds or human made constructs that can imitate or interfere with endogenous and endocrine activities of hormones (49). These compounds with estrogenic, androgenic, anti-estrogenic or anti-androgenic effects are abundant in some human-made compounds, including plastic products, cosmetic products, drink bottles, disposable utensils, dental equipment as well as in herbal compounds, including phytoestrogens, are found (50, 49). Until now, some human-made compounds or natural compounds have been identified as having an disruptive activity on HPG and reproductive function. Human made compounds include some of the insecticides, bisphenol A, phthalates, and chemical drugs such as tamoxifen and natural compounds, including phytoestrogens and fungal estrogens (Mycostrogens) (53-51).

Studies have shown that EDCs with steroid-like activities, in addition to their effects on the different

levels of HPG axis, including pituitary and gonads, may also interfere with the inappropriate gender differentiation of neural networks, including the kisspeptin system (57-54, 50, 49). Recent studies have shown that the kisspeptin system is a major and dominant target for estrogenic EDCs, which leads to various reproductive disorders in mammals (52, 49). These studies have shown that the evolution of the kisspeptin system during growth and development, especially during the infancy, is highly sensitive to estrogen and estrogenic EDCs. So that any changes in the estrogen content of the rodent baby result in pivotal and permanent changes at the onset of puberty, GnRH/gonadotropin disruption and disturbance, estrus cycle dysfunction, and ultimately reproductive impairment (57, 49).

Other studies have shown that the administration of neonatal Myco-estrogen Zearalenone in female mice is able to disrupt kisspeptin signaling and reduce neuronal density in ARC and AVPV hypothalamic nuclei, and ultimately causes early sexual maturity, estrus cycle dysfunction and decreased ovarian follicles (59, 58).

Patisaul et al. demonstrated that the administration of estrogenic compounds (propylpyrazole triol=PPT), bisphenol A, and phytoestrogens during the first 4 days after birth reduced the number of kisspeptin neurons, resulted in the disorder in the organization of kisspeptin fibers and, consequently, reproductive impairment In adult rats (60). Losa et al. showed that neonatal treatment of female rats with genistein causes disruptions in the growth of kisspeptin signaling pathways and also deficiency in the development of ovaries in mature rats (61).

Hu and his colleagues also showed that the treatment of female mice with Dibutyl phthalate in neonatal and pre-puberty periods, led to premature puberty and interruption of the GPRG54 and kisspeptin expression (62). It was also shown in a study that prescribing tamoxifen (a modifying agent for estrogen receptors in the treatment of breast cancer) in female baby mice in the first 5 days after birth reduced the expression of kisspeptin and reduced neuronal density in AVPV and ARC nuclei in combination with accelerated vaginal opening (symptoms of puberty), Estrous cycle disorder, decreased ovarian follicle content profile, decreased yellow bodies, increased number of departed follicles, and disturbances in estradiol and LH secretion in adult mice (63).

## Discussion

Taken together, the results presented here indicate that kisspeptin signaling is essential for regulating the onset of puberty, proper reproductive performance, and successful fertility. Kisspeptin neurons act as a central focal point for receiving peripheral and internal messages in the hypothalamus, and then converting these messages into GnRH neurons, but the function of

these messages on the kisspeptin neurons is still not well understood. Studies have also shown that the kisspeptin system, especially during the neonatal period, is highly sensitive to estrogen-like and endocrine-induced endocrine changes, as many reproductive disorders and infertility may be due to disruption of the kisspeptin system.

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