Review Article

Kisspeptin: A Novel Regulator in Reproductive Physiology

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Abstract

Kisspeptin (a product of the Kiss1gene) and its receptor (GPR54 or Kiss1r) have emerged as key players in regulation of reproduction. Ever since the discovery of kisspeptin, intensive studies on hypothalamic expression of KISS1/Kiss1 and on physiological roles of hypothalamic kisspeptin neurons have provided clues as to neuroendocrine control of GnRH neurons by orchestrates the sequences that take place during oestrous cycle, onset of puberty, and control of fertility by upstream of GnRH and have been shown to play a vital role in the control of hypothalamic–pituitary–gonadal axis via regulation of gonadotrophin secretion.. Additionally, emerging evidence indicates the potential involvement of extra-hypothalamic kisspeptin in reproductive functions. Kisspeptin signaling may also serve diverse functions outside of the classical realm of reproductive neuroendocrinology, including the regulation of metastasis in certain cancers, vascular dynamics, placental physiology, and perhaps even higher order brain function. Hence, kisspeptins have potential diagnostic and therapeutic applications.

Key words: GnRH, Kisspeptin, Reproduction, Therapeutic Application

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Introduction

Kisspeptin was discovered as a metastasis-suppressor gene in 1996 (Lee *et al.,* 1996). KISS1 was named for its role as a suppressor sequence (ss); the letters "KI" were appended to the prefix "SS" to form "KISS" in homage to the location of its discovery, Hershey, Pennsylvania, home of the famous "Hershey Chocolate Kiss." Kisspeptins are a family of structurally related peptides, encoded by the KISS1/Kiss1 gene, that act through binding and subsequent activation of the G protein-coupled receptor GPR54. Hypothalamic kisspeptin neurons are mainly localized in two regions: the anterior region of the hypothalamus called the anteroventral periventricular nucleus (AVPV) in rodents, or the preoptic area (POA) in other species and the posterior region of the hypothalamus called the arcuate nucleus (ARC). Kisspeptin neurons are sexually

differentiated with respect to cell number and transcriptional activity in certain brain nuclei, and some kisspeptin neurons express other co-transmitters, including dynorphin and neurokinin B. It has been implied that via the inhibitory action of dynorphin and the stimulatory action of neurokinin B, KNDy neurons regulate kisspeptin secretion, which further modulates pulsatile release of GnRH and LH (Navarro *et al*., 2009), which plays a pivotal role in controlling the onset of puberty and reproduction in both sexes and further Kisspeptin agonists and antagonists have potential diagnostic and therapeutic applications.

Major Structural Feature of Kisspeptin

Kisspeptins are derived from the differential proteolytic processing of a single precursor. In the human, the kisspeptin precursor comprises 145 amino acids, with a putative 19-amino acid signal sequence, two potential dibasic cleavage sites (at amino acids 57 and 67), and one site for terminal cleavage and amidation (at amino acids 121–124) (Kotani *et al.*, 2001; Ohtaki *et al.*, 2001), which generates the biologically active kisspeptins. Indeed, proteolysis of prepro-kisspeptin gives rise to a 54-amino acid peptide (kisspeptin-54), initially termed metastin because of its capacity to inhibit tumor metastasis, which has been considered the major product of the KISS1 gene (Ohtaki *et al.*,2001). In addition, other peptide fragments of the kisspeptin precursor have been identified, such as kisspeptin-14, kisspeptin-13, and kisspeptin-10 (Bilban *et al*., 2004; Kotan *et al.*,2001), that share the COOH-terminal region of the kisspeptin-54 molecule, where they harbor an Arg-Phe-NH_2 motif characteristic of the RF-amide peptide family (Fig. 1).

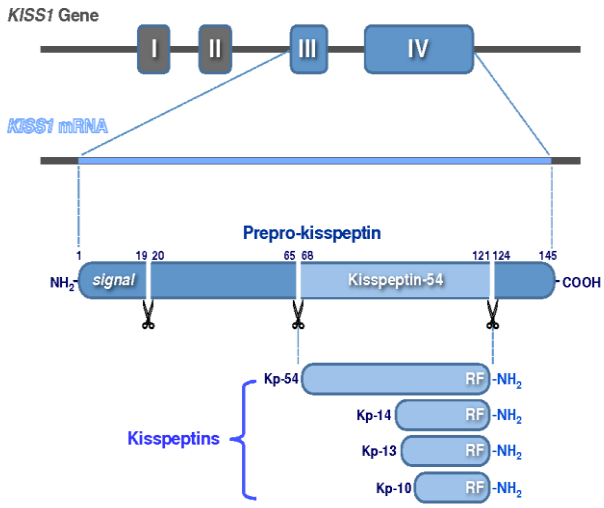

Fig. 1: Major structural features of kisspeptins, the products of the Kiss1 gene (Roa *et al.*, 2008)

Expression of Kisspeptin and GPR54

Kisspeptin and GPR54 have been found within the hypothalamus, brainstem, spinal cord, pituitary, ovary, prostate, liver, pancreas, intestine, aorta, coronary artery, umbilical vein and placenta (Lee *et al.*, 1996; Ohtaki *et al.*, 2001; Mead *et al.*, 2007; Richard *et al.*, 2008; Roseweir and Millar, 2009).

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Table 1: Effect of Kisspeptin on different organ (Matvienko *et al*., 2013)

Area of Influence	Effect	Species
Hypothalamus	Gonadotropin releasing (GnRH)	Sheep, Goat, pig
GnRH-neurons	Depolarization, increasing pulsation	Human, rat
Pituitary	FSH and LH releasing	Human, rat
Epiphysis	Stimulation of melatonin synthesis in young and mature animals, depression – in the old ones	Rat
Hippocampus	Neural transmission	Rat
Alpha-adrenergic system	Strong activation in young and mature organism and neutral effect in old one	Rat
Placenta	Prevents the trophoblast migration, regulates the gonadotropic axis activation in the fetus	Human
Heart	Positive inotropic effect	Human, mouse
Aorta, umbilical vein	Strong vasoconstriction	Human
Pancreas	Affects the insulin secretion	Human, rat
Testes	Enhances the secretory activity, pancreases /testosterone production	Rat
Ovaries	Activating of estrogen releasing	Human, rat
Skin, thyroid, ovaries, bladder, breast, stomach, esophagus, liver, pancreas, lung, prostate	Metastasis supression	Human, rat

Cellular Action of Kisspeptins on GnRH Neurons

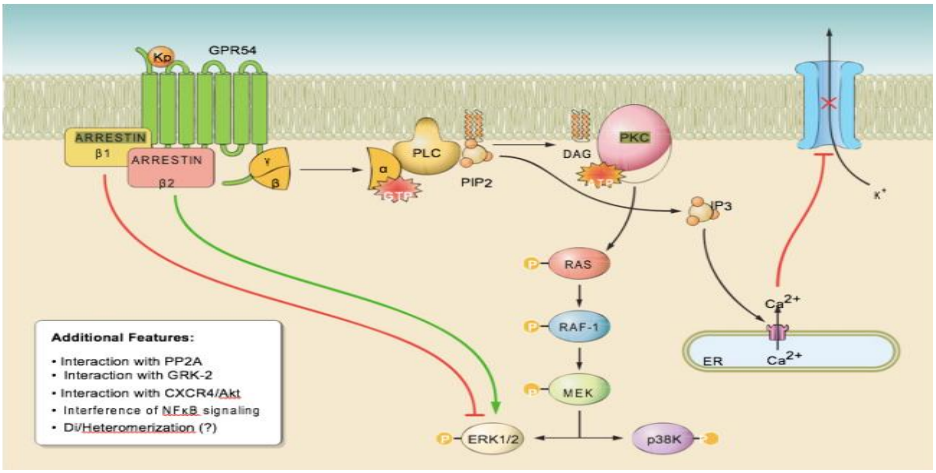

Fig. 2: Major signaling pathways recruited upon GPR54 activation by kisspeptins (Castano *et al*., 2009; Roa *et al*., 2009)

GPR54 is a seven transmembrane domain, Gq/11-coupled receptor, whose activation leads to increases in intracellular Ca^2 levels [Ca^2]i in a pertussis toxin-independent manner, without detectable changes in intracellular cAMP levels, therefore suggesting the lack of association with Gs and/or Gi/o proteins (Kotani *et al.,* 2001). This increase in [Ca^2]i is caused by the activation of phospholipase C (PLC), with the subsequent stimulation of the hydrolysis of phosphatidyl-inositol bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3), which in turn evokes the mobilization of this ion from intracellular stores. Such an

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increase in phosphatidyl inositol turnover has been demonstrated for both human and mouse GPR54 (Kotani *et al.*, 2001; Stafford *et al.*, 2002). In addition, the rise of PIP_2 hydrolysis following kisspeptin stimulation leads to diacylglycerol (DAG) formation and thereby, protein kinase C (PKC) activation (Ringel *et al.*, 2002). In turn, activated PKC is thought to cause phosphorylation of mitogen-activated protein kinases (MAPKs), such as ERK1/2 and p38, which have been also involved in this signaling cascade (Kotani *et al.*, 2001). In addition, activation of GPR54 has been reported to increase arachidonic acid release in CHO-K1 cells stably expressing this receptor (Kotani *et al.*, 2001). From a physiological perspective, it is worth nothing that studies using hypothalamic explants and isolated GnRH neurons have fully confirmed the importance of the above PLC-Ca^2 pathway in mediating the biological effects of kisspeptins in a more relevant cellular context in terms of control of reproductive function, such as hypothalamic explants and GnRH neurons (Castellano *et al.*, 2006; Liu *et al.*, 2008). The above signaling features do not only have implications in terms of regulation of hormone secretion and neuroendocrine function, but are also the basis for additional biological actions of kisspeptins, such as the control of cell proliferation and migration. Thus, as mentioned above, activation of GPR54 leads to phosphorylation of different MAPK, which might contribute to the antimetastatic and/or antiproliferative effects of kisspeptins (Castano *et al.*, 2009).

Physiological Role of Kisspeptins in Modulating GnRH Secretion

The two major populations of kisspeptin neurons localized in the POA/AVPV and ARC are considered to have separate roles in female reproduction, because earlier studies in rodents demonstrated a different pattern of Kiss1 expression in these two hypothalamic regions.

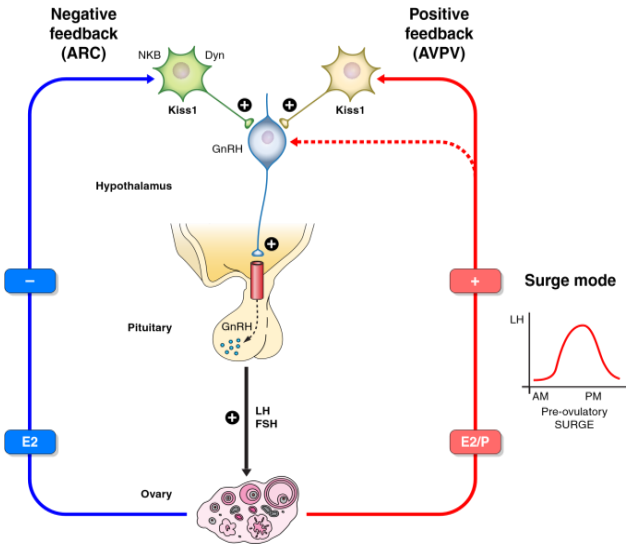

Fig. 3: Differential regulation and actions of ARC vs. AVPV Kiss1 neurons in the control of GnRH in rodents (Tena-Sempere, 2010)

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The AVPV kisspeptin neurons are a target of estrogen positive feedback action and hence generate the GnRH surge and that the ARC kisspeptin neurons are a target of estrogen negative feedback action and are involved in GnRH pulse generation. Kisspeptin immunoreactive fibers originating from cell bodies in the ARC make close apposition to GnRH axons in the median eminence of the monkey (Ramaswamy *et al.*, 2008) and are proposed to modulate the pulsatile release of GnRH and act through action on HPG axis.

Role of Kisspeptin in Reproductive Physiology

Kisspeptin and Pituitary

Interestingly, kisspeptins have been identified in the ovine hypophyseal portal blood (Smith, 2008), leading to the proposition that kisspeptin may act at the level of the pituitary to directly induce LH secretion from the gonadotropes. GnRH antagonist inhibits the typical kisspeptin-induced increase in LH (Gottsch, 2004; Irwig, 2004), indicating that the primary actions of kisspeptin on gonadotropin secretion occur upstream of the pituitary. Functional studies focusing on the direct stimulatory effects of kisspeptin on pituitary gonadotropin secretion have yielded conflicting results and suggest that kisspeptin cannot independently prompt the LH surge. It is therefore likely that at the pituitary, kisspeptin acts synergistically with GnRH and estradiol to stimulate gonadotropin secretion.

Kisspeptin and Gender Differentiation

There are also differences in various developmental stages of the animal's life, which indicates different upstream pathways, including Kisspeptin system, converging upon GnRH neurons (Kauffman, 2010). The Kisspeptin system is apparently critical for brain gender differentiation, acting through the regulation of postnatal T secretion. Anatomical differences between genders have been reported in the hypothalamus of some species, e.g. the rat AVPV is sexually dimorphic, with a greater number of KISS1 neurons in females compared to males (Kauffman *et al.*, 2007).

Kisspeptin and Onset of Puberty

Timing of puberty onset is determined by genetic and environmental factors as well as gene-environment interactions, and is effectively different between males and females. It has been shown that puberty will not occur without proper interaction of Kisspeptins and their corresponding receptor, e.g. inactivating mutations of GPR54 gene in hypogonadotropic hypogonadism subjects (Funes *et al.*, 2003; de Roux *et al.*, 2003; Seminara *et al.*, 2003). Hypothalamic Kiss1 system participates in the control of puberty onset and is likely to include, at least four major related components:

1) an elevation in the endogenous kisspeptin tone, which seems to be sufficient per se to drive the GnRH/gonadotropin axis to a state of full activation,
2) an increase in the sensitivity to the stimulatory effects of kisspeptin in terms of GnRH/LH responses,
3) an enhancement of GPR54 signaling efficiency, which is apparently coupled to a state of resistance to

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desensitization to kisspeptin stimulation, and

4) an increase in the number of kisspeptin neurons, at the AVPV and/or the ARC depending on the species, as well as of their projections to GnRH neurons, originating mainly from the AVPV in rodents (Pinilla *et al.*,2012).

Maturational Changes of Kiss1 System during Female Puberty

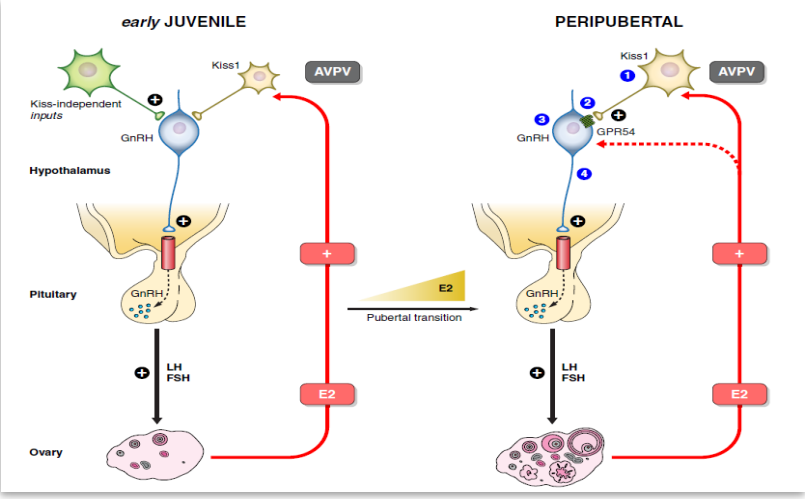

Fig. 4: Maturational changes of Kiss1 system during female puberty (Roa and Tena-Sempere, 2010; Tena-Sempere, 2010)

Endogenous Kisspeptin rhythmicity and sensitivity to it increases at the time of puberty; in primates and rats, an increase in both the number of KISS1 neurons and the content of Kiss1 mRNA has been reported during juvenile-pubertal transition (Kauffman, 2010; Mayer *et al.*, 2010; Shahab *et al.*, 2005).

Action of Kisspeptin on Ovary

Locally produced ovarian kisspeptin directly influences folliculogenesis, ovulation, and perhaps luteal function in rats, which may also apply to other animals, including humans and marmosets, where kisspeptin has been identified in the ovary (Kalamatianos *et al.*, 2008). Gpr54 mRNA levels remained rather low and stable across the ovarian cycle, ovarian Kiss1 expression increased during the pubertal transition and peaked at the afternoon of proestrus, i.e., preceding ovulation (Castellano *et al.*, 2006). Recent studies in the Siberian hamster demonstrated enhanced kisspeptin-IR during the ovulatory transition, i.e. proestrus and estrus (Shahed and Young, 2009). Ovarian expression of Kiss1 appears to be under the regulation of pituitary gonadotropins, since protocols of gonadotropin priming were able to enhance Kiss1 mRNA levels in the ovary of immature rats, while prevention of the preovulatory surge of gonadotropins blocked the rise of ovarian Kiss1 expression (Castellano *et al.*, 2006).

Kisspeptin Level in Pregnancy

Circulating kisspeptin levels are low in males and non-pregnant females (<2pmol/l) but dramatically

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increase in pregnancy (Horikoshi *et al.,* 2003). In the first reported study of 10 pregnant women and 12 non-pregnant controls, kisspeptin levels increased by 940-fold in the first trimester and further increased to some~7000-fold higher in the third trimester. Circulating kisspeptin levels fell again 5days post-delivery to comparable concentrations prior to pregnancy, implicating a placental source of the peptide. Kisspeptin has a prime location at the foeto-maternal interface, being abundant in the syncytiotrophoblast of the both normal human placenta (Bilban *et al.,* 2004) and in molar pregnancies (Janneau *et al.,* 2002). The outer syncytiotrophoblasts lie adjacent to blood vessels allowing easy passage of kisspeptin into the maternal blood. KISS1 mRNA is expressed in the trophoblast giant cells of the rodent placenta (Terao *et al.,* 2004), which are responsible for early invasion as they invade the spiral arteries and replace the endovasculature.

Kisspeptin in Lactation

Lactating rats have reduced expression of Kiss1 mRNA in the ARC region and Kiss1r mRNA expression in the AVPV (Yamada *et al.,* 2007), providing a possible mechanism to explain the reduction of LH secretion during lactation. The suckling stimulus appears to be responsible for the suppression of Kiss1 mRNA expression in the ARC (Yamada *et al.,* 2007).

Action of Kisspeptin on Male Reproductive Tract

Kisspeptin and its receptor have been suggested to be involved in the regulation of human sperm motility and male fertility. It has been evidenced by detection of kisspeptin and its receptor in human sperm, which could be activated by kisspeptin treatment while sperm activity was blocked by kisspeptin antagonists (Hoffman *et al.,* 2011). Similarly, Kiss1 and Kiss1r have been detected in the testes of mice and have been suggested to regulate sperm function, although kisspeptins failed to release testosterone form seminiferous tubule explants (Homma *et al.,* 2009; Horikoshi *et al.,* 2003).

A Photoperiodic Role for KiSS-1 in Seasonal Breeding

Kisspeptin is the missing link between melatonin and the HPG axis. So far, it was clear that melatonin acts on a system distinct from the GnRH neurons (or upstream of these neurons). Several arguments suggest a role for KiSS-1/GPR54 in the photoperiodic control of reproduction. First, the sexual phenotype of GPR54 loss-of-function mutant mice remarkably resembles the phenotype of Syrian hamsters exposed to SD (low circulating gonadotropins, atrophied gonads and low levels of sex steroids); both photo-inhibited hamsters and mutant mice have normal GnRH expression, and GnRH administration is still able to trigger LH/FSH release. Second, some KiSS-1 cells are found in the MBH, precisely where the physiological target sites for melatonin action on reproduction are thought to reside. The finding that photoperiod modulates KiSS-1 expression via melatonin strongly suggests that kisspeptin relays photoperiodic information to the HPG

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axis, and that reduced kisspeptin signaling in SD hamsters may be responsible for the inhibition of reproductive activity.

Role of the Kisspeptin Synergism with GnRH and Estradiol in Dairy Animals

Kisspeptin clearly stimulates release of GnRH and sub-sequent secretion of LH. It has proved that, Kp-10 increases circulating concentrations of LH in pre-pubertal male and female Japanese Black calves (Ezzat *et al*., 2010). Kisspeptin-10 also stimulates increased circulating concentrations of LH in Holstein cows and ovariectomized Jersey cows, and interestingly the sensitivity of LH to exogenous Kp-10 stimulation seems to be enhanced with lactation (Whitlock *et al*., 2010; Whitlock *et al*., 2011). One study showed that Kp10 treatment stimulates LH secretion from anterior pituitary cells in bovines (Ezzat *et al.,* 2010). In cattle, kisspeptin along with luteinizing hormone (LH), also excites growth hormone (GH) in ovariectomized cows, which were injected with kisspeptin10 (Kp10) in different doses. In vitro analysis indicated that kisspeptin is relevant to the release of growth hormone (GH) and prolactin (PRL) as well as the release of gonadotropin in ruminants (Hashizume *et al.,* 2010). In small ruminant like in adult ewe neurons, KiSS-1 mRNA has been found to rise in the caudal Arc during the follicular phase and in the rostal Arc at estrus when the LH surge occurs (Estrada *et al.,* 2006). This suggests that the Arc is responsible for modulating the positive steroid feedback in the ewe. In the intact ewe, during the estrus cycle, kisspeptin can synchronize LH surges and during anoestrus, administration of kisspeptin can cause ovulation to occur, suggesting that when kisspeptin levels are high enough they can cause the LH surge and are therefore probably involved in relaying positive feedback to GnRH neurons (Caraty *et al.,* 2007).

Therapeutic Application of Kisspeptin

Kisspeptins and neurokinin B (NKB) provide a novel therapeutic approach for treating disorders with either pathologically reduced or augmented gonadotrophin pulsatile secretion. Kisspeptin and NKB agonists may be used to stimulate the HPG axis in conditions with reproductive insuficiency of central origin provided the GnRH neuronal system is intact. It has been suggested that kisspeptins might be associated with less risk of ovarian hyperstimulation syndrome (OHSS) as compared to routinely used hCG injections, and further work is now underway in a large population who are at high risk of OHSS (Abbara *et al.,* 2014). Kisspeptin antagonists might also be helpful in normalising relative LH hypersecretion with subsequent improved follicular development and ovulation in patients having polycystic ovary syndrome (PCOS) (Skorupskaite *et al.,* 2014; McNeilly *et al.,* 2003). In a recent randomized trial researcher found that NKB antagonist (AZD4901) administration in patients having PCOS resulted in reduced LH pulse frequency and secretion with subsequent remarkable and sustained reduction in testosterone levels (George *et al.,* 2015). Likewise, kisspeptin and NKB antagonists might be helpful in treating patients having precocious puberty (Skorupskaite *et al.,* 2014).

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Table 2: Effects of synthetic KiSS1R agonists on hormone concentration in livestock

Species	Sex	Status	Molecule	Administration Route	Dose	Effect on LH	Effect on FSH	Effect on testosterone	References
Sheep	Female	Adult Non-cyclic	FTM080	iv	0.5,2.5 or 5 nmol/ kg	Short increase (at all doses)			(Whitlock *et al.*,2015)
	Female	Adult Non-cyclic	Compound 17	iv	15 nmol/ewe	Increase lasting about 9 h	Increase during approximately 5 h		(Beltramo *et al.*,2015)
	Male	Adult	C6	im	15 nmol/ram	Increase lasting about 10 h		Induces prolonged testosterone secretion	(Decourt *et al.*,unpublished)
	Female	Adult	C6	im	15 nmol/ewe	Increase lasting about 12 h	Biphasic release		(Decourt *et al.*,2016)
	Female	Adult Follicular phase	C6	im	15 nmol/ewe	Increase lasting about 12 h	Increases during approximately 10 h		(Decourt *et al.*,2016)
Goat	Female	Adult Non-cyclic	TAK-683	Sc infusion	50 nmol/kg/ week	Abolish pulsatility			(Tanaka *et al.*,2013)
						Unable to block estradiol-induced LH surge			
	Female	Adult OVX	TAK-683	iv	35 nmol/ goat	Rapid increase			(Goto *et al.*,2014)
	Female	Adult Follicular phase	TAK-683	iv	35 nmol/ goat	Increase lasting about 12 h	Immediate decrease		(Goto *et al.*,2014; Endo *et al.*,2015)
	Female	Adult luteal Phase	TAK-683	iv	35 nmol/ goat	Increase pulse frequency	Increase sufficiently to trigger an LH surge		(Goto *et al.*,2014; Endo *et al.*, 2015)

Future Prospects of Kisspeptin

Fundamental research has identified kisspeptin and the neurokinins as principle regulators of diverse aspects of reproductive physiology and pathology. These include both common disorders such as PCOS, as well as more rare conditions such as idiopathic hypogonadotrophic hypogonadism. To aim central reproductive and non-reproductive pathways, agonists and antagonists for kisspeptin and NKB receptors may require accessing the brain and crossing the blood-brain-barrier (BBB). Along with, appropriate doses and routes of administration need to be specified for the current and future arsenal of agonists, antagonists and mixed agonists-antagonists. Altogether, our understanding of the physiological basis, and eventual physiopathological implications of kisspeptin signaling in the brain will help in overcoming the challenges in drug development for several reproductive disorders *viz.* hypothalamic amenorrhoea, hyperprolactinaemia, infertility, menopausal hot flushes, psychosexual disorders and PCOS. It is further

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anticipated that additional progress will be made towards the characterization of kisspeptins as targets for pharmacological intervention of the reproductive system.

Conclusion

Kisspeptin is a peptide with a diverse and multifunctional nature, involving varied whole body physiological systems and acting at all levels of the reproductive axis-brain, pituitary, gonad, and accessory organs. Kisspeptin exercises a crucial role in stimulating GnRH, relaying steroid hormone negative and positive feedback signals to GnRH neurons, serving as a gatekeeper to the onset of puberty and also involved in other reproductive functions and the manipulation of pulsatile release of GnRH has been suggested to have a therapeutic potential for future development of drugs that might control reproduction.

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