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The role of kisspeptin signalling in the hypothalamic–pituitary–gonadal axis — current perspective

Rola sygnalizacji kisspeptyny w osi podwzgórze–przysadka–nadnercza — aktualna perspektywa

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Abstract

The discovery of kisspeptins in the recent past remoulded current understanding of the neuroendocrine axis relating to the regulation of human puberty and reproduction. Kisspeptins have been recognised to act upstream of GnRH and have been shown to play a vital role in the control of the hypothalamic–pituitary–gonadal axis via regulation of gonadotrophin secretion, onset of puberty, and control of fertility. KNDy (kisspeptin/neurokinin-B/dynorphin) neurons have been suggested to modulate GnRH pulsatile secretion, which is required to support reproductive function in both sexes. They have also been involved in mediating both positive and negative sex steroid feedback signals to GnRH neurons and serve as a vital connection between reproduction and metabolic status of the body. When kisspeptin is administered to healthy humans, and in patients with reproductive disorders, it strongly and directly stimulates GnRH and subsequent LH secretion and enhances LH pulse frequency. These observations suggest that kisspeptins are a potential novel therapeutic approach for treating disorders with either pathologically reduced or augmented gonadotrophins pulsatile secretion and is currently a focus of translational research. Kisspeptins have also been identified in several peripheral reproductive organs, indicating their role in modulation of ovarian function, embryo implantation, and placentation, but a great deal of work remains to be done to explore further in this regard, and the evidence is only available from studies done on animal models. In this review we will mainly focus on current available evidence related to the role of kisspeptins in controlling GnRH pulse frequency, specifically their role in puberty, fertility, and reproduction. We will also be appraising other factors that regulate the kiSS1/Kisspeptin/GPR-54 system. **(Endokrynol Pol 2015; 66 (6): 534–547)**

Key words: *Kisspeptin signalling; hypothalamic–pituitary–gonadal axis; gonadotrophin secretion; puberty; reproduction*

Streszczenie

Odkrycie kisspeptyn, które miało miejsce całkiem niedawno, odmieniło obecne rozumienie osi neuroendokrynnej, związanej z regulacją okresu dojrzewania i rozrodu. Odkryto, że kisspeptyny działają przed GnRH i odgrywają istotną rolę w kontroli osi podwzgórze–przysadka–nadnercza poprzez regulację wydzielania gonadotropiny, rozpoczęcia okresu dojrzewania oraz kontroli płodności. Zasugerowano, że komórki KNDy (kisspeptyna/neurokinina-B/dynorfina) modulują pulsacyjne uwalnianie GnRH, wymagane, aby wspomagać funkcję rozrodczą u obu płci. Komórki te są również zaangażowane w przekazywanie zarówno pozytywnych, jak i negatywnych sygnałów hormonów płciowych do neuronów GnRH, a także stanowią kluczowe połączenie między reprodukcją i stanem metabolicznym ciała. Kiedy kisspeptyna jest podawana jednostkom zdrowym i pacjentom z zaburzeniami płodności, silnie i bezpośrednio stymuluje GnPH i dalsze uwalnianie LH oraz poprawia częstotliwość impulsów LH. Obserwacje te przedstawiają kisspeptyny jako nowe potencjalne terapeutyczne podejście w leczeniu zaburzeń patologicznie obniżonego lub zwiększonego pulsacyjnego uwalniania gonadotropin i obecnie stanowi główny punkt zainteresowania badań przekładających się na zastosowanie praktyczne. Kisspeptyny zidentyfikowano także w kilku organach obwodowych, uczestnicząc w modulacji czynności jajników, implantacji zarodka oraz placentacji, lecz dalsze badania w tym kierunku będą wymagały jeszcze wiele wysiłku, a dowody można uzyskać jedynie z badań przeprowadzanych na modelach zwierzęcych. W niniejszej pracy autorzy skupili się głównie na obecnie dostępnych dowodach związanych z rolą kisspeptyn w kontrolowaniu częstotliwości impulsów GnRH, a zwłaszcza ich rolą w okresie dojrzewania, płodności oraz reprodukcji. W niniejszym artykule poddano ocenie także inne czynniki regulujące system kiSS1/Kisspeptin/GPR-54. **(Endokrynol Pol 2015; 66 (6): 534–547)**

Słowa kluczowe: *sygnalizacja kisspeptyny; oś podwzgórze–przysadka–nadnercza; uwalnianie gonadotropiny; okres dojrzewania; rozród*

Introduction

It is well established that secretion of gonadotrophic releasing hormone (GnRH) from the hypothalamus is the key pathway that commences and controls reproductive function [1]. But some functional limitations in this pathway have been identified that lead to the suggestion that there must be some additional intermediate pathway controlling the hypothalamic-pituitary-gonadal (HPG) axis. One of them was the absence of oestrogen receptors (ER-α) in GnRH neurons in rats, which suggested some upstream pathway mediating gonadal feedback [2].

The discovery of kisspeptins in the recent past remoulded current understanding of the neuroendocrine

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axis related to regulation of human puberty and reproduction [1, 3]. Kisspeptins have been shown to play a vital role in the control of the HPG-axis via regulation of GnRH secretion, and their discovery set the foundation for further advances in untwisting the complexities of peripheral and central regulation of reproduction [4, 5]. Kisspeptin is a hypothalamic peptide that is encoded by KiSS1 gene [6]. It has been recognised to act upstream of GnRH and to be a pivotal regulator of gonadotrophins secretion, onset of puberty, and control of fertility [7]. More recent evidence has shown the involvement of other factors in controlling reproduction, in addition to kisspeptins, including neuropeptides such as neuropeptide Y (NPY) and nesfatin-1 [8, 9], and neurokinin B (NKB) [10]. The neuronal network that secretes neurokinin B and kisspeptin is made up of KNDy (Kisspeptin-neurokinin B dynorphin) neurons as they also produce dynorphin [1, 10, 11].

In this review we will focus on current available evidence related to the role of kisspeptin in controlling GnRH pulse frequency, specifically its role in puberty, fertility, and reproduction. We will also appraise other factors that regulate the kiSS1/Kisspeptin/GPR-54 system.

Methods

An extensive search of PubMed was performed for all the articles published up to March 2015 related to the kiSS1/Kisspeptin/GPR-54 system. The search was performed mainly for the articles related to human studies, but studies related to other species were also reviewed and where appropriate were included.

Discovery of KiSS1/kisspeptin/GPR54

The KiSS1 gene which encodes peptide products, kisspeptins, was originally discovered in 1996 as a metastasis suppressor gene in malignant melanoma cells [6]. It was named after the famous Hershey's chocolate 'kisses' as it was first discovered in Hershey, Pennsylvania, USA. The KiSS1 gene is located on human chromosome 1 (1q32), which initially produces a 145 amino acid precursor peptide (prepro-kisspeptin), which is cleaved to 54 amino acid protein (kisspeptin-54, Kp-45, formerly called metastin) [12, 13]. Kp-54 may be further cleaved to lower molecular weight forms of kisspeptins, Kp-14, Kp-13, and Kp-10, sharing a common C-terminal sequence of arginine-phenylalanine-NH2 motif that is sufficient to fully activate GPR54. These peptides are collectively now recognised as kisspeptins [4, 14, 15].

GPR54 (G-protein coupled receptor 54), now termed KiSS1R, was first discovered in the rat brain in 1999 as an orphan receptor [16] and later in humans, and was named AXOR12 or hOT7T175 [13, 17]. In 2001 GPR54 was categorised as a putative receptor for kisspeptins [13, 17].

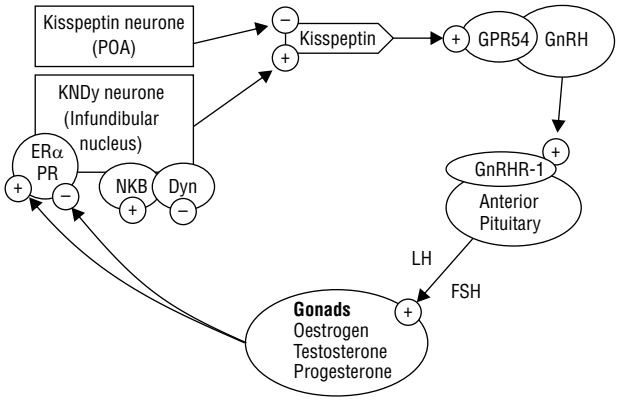
Discovery of the reproductive role of kisspeptins

The role of KiSS1 in reproduction remained unrecognised until the end of 2003 when two independent groups discovered that mutations of GPR54 were associated with idiopathic hypogonadotrophic hypogonadism (iHH) [3, 5]. These findings in humans were subsequently found in animal studies in which GPR54 knockout (KO) mice showed small testis and ovaries, low gonadotrophins, delayed puberty, and reduced fertility and sexual behaviour [5, 18]. These findings were confirmed more recently in KiSS1 and GPR54 KO mice models that developed features of iHH, although the features were more severe in GPR54 KO mice [19–21]. These discoveries identified kisspeptins and GPR54 as pivotal regulators of key aspects of puberty and reproductive function and paved the way for further exploration to unravel the underlying mysteries related to the reproductive actions of kisspeptins.

Functional neuroanatomy of the kisspeptin system

Studies have revealed that the location of kisspeptin neurons within the hypothalamus is species specific, but we will be focusing mainly on current understanding of human neuroanatomy with evidence from animal studies if needed. Several studies have localised kisspeptin neurons in the infundibular/arcuate nuclei across all species, including humans, and have suggested that the rostral portion of the hypothalamus is species specific [22–26] (Fig. 1) [27, 28]. The studies performed on human autopsy samples recently have confirmed that kisspeptin neurons in humans are mainly located in the infundibular (arcuate in other species) and rostral preoptic area (POA) of the hypothalamus [24, 29]. Studies have also indicated direct participation of kisspeptin in GnRH secretion [30], as evidenced by kisspeptin receptor mRNA expression by GnRH neurons [31, 32], and close connections found between kisspeptin and GnRH neurons [22, 24, 26], although in humans not all GnRH neurons showed close connection with kisspeptin neurons [22, 24, 26, 33].

Later it was discovered that kisspeptin neurons located in the infundibular region in humans/arcuate nucleus in rodents co-express other neuropeptides, named neurokinin-B and dynorphin, and were collectively called KNDy neurons [24, 29, 34], but neurons in POA did not express any of these neuropeptides [1]. It has been suggested that these distinctive populations of kisspeptin neurons also differ in physiological function [35, 36]. The KNDy neurons autosynaptically modulate pulsatile secretion of kisspeptin and GnRH



POA — preoptic area of hypothalamus; KNDy neuron — kisspeptin neurokinin dynorphin neuron; GPR54 — G-protein coupled receptor 54 (GPCR); GnRH — gonadotrophin releasing hormone; GnRHR-1 — gonadotrophin releasing hormone receptor 1, LH — luteinizing hormone, FSH — follicle stimulating hormone, ERα — oestrogen receptor alpha, PR — progesterone receptor, NKB — neurokinin-B, Dyn — dynorphin, + stimulatory, – inhibitory

Figure 1. *Diagrammatic representation of the relationship between kisspeptin neurons, KNDy neurons, and GnRH neurons in humans. Adapted from Dungan et al., 2006; Gottsch et al., 2006; Oakley et al., 2009; Roseweir and Millar, 2009; Skorupskaite et al., 2014*

Rycina 1. *Schemat relacji między neuronami Kisspeptin, neuronami KNDy oraz neuronami GnRH u ludzi. Na podstawie: Dungan i wsp., 2006; Gottsch i wsp., 2006; Oakley i wsp., 2009; Roseweir and Millar, 2009; Skorupskaite i wsp., 2014*

[10, 37], as evidenced by expression of dynorphin receptors (kappa opioid peptide receptor) and neurokinin-B receptors by KNDy neurons [38, 39]. 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 [38]. It has also been suggested that these KNDy neurons in the infundibular region in humans are mainly involved in relaying both positive and negative sex steroid feedback signalling [29, 40].

The evidence has also shown sexual dimorphism in kisspeptin neuron pathways in humans and in other species, with obvious differences in expression of both kisspeptin fibres and cell bodies in hypothalamus nuclei [24, 34], further explaining their differential physiological functions.

Physiological role of kisspeptins in modulating GnRH secretion

Evidence suggests that kisspeptin acts directly on the GnRH neurons and stimulates the release of GnRH after interaction with its receptor. GnRH further stimulates gonadotrophs in the pituitary gland to secrete FSH and LH into the peripheral circulation [1]. The evidence related to the stimulatory effects of kisspeptins on GnRH neurons has been provided mainly from animal studies [7]. They have shown increased expression of GnRH mRNA by GnRH neurons after kisspeptin exposure [40, 41]. Likewise, it has been shown that kisspeptins can evoke an increased firing rate of GnRH neurons as measured by voltage recordings in hypothalamic slices from mice [42–45], stimulate the release of GnRH in hypothalamus explants [46, 47], and cause a dramatic increase in GnRH in the CSF of sheep [48]. Studies using kisspeptin antagonist have further elaborated its role in modulating GnRH secretion, by demonstrating that increased firing of GnRH neurons induced by kisspeptin was eliminated by kisspeptin antagonist [44, 49, 50].

Few other studies have suggested that kisspeptin stimulates the pituitary gland directly to release FSH and LH. It has been shown from animal studies revealing expression of genes related to kisspeptin and its receptor in gonadotrophs[51], secretion of FSH and LH from kisspeptin treated pituitary explants [52, 53], and the presence of kisspeptin in the hypophyseal portal system [54]. It has been further explained that although kisspeptin may directly stimulate the pituitary gland, the evidence is more in favour of indirect stimulation of gonadotrophs by increasing GnRH secretion as the principal physiological pathway [1].

The role of kisspeptins in GnRH pulse generation

The manipulation of pulsatile release of GnRH has been suggested to have a therapeutic potential for future development of drugs that might control reproduction [55]. Kisspeptin neurons have been suggested as a GnRH pulse generator that is required to support the reproductive function in both sexes, such as follicular development, sex steroid production, and spermatogenesis [55]. This possibility has been supported by anatomical, functional and recent pharmacological data from both humans and animals [7]. The evidence suggests the arcuate nucleus as the site of GnRH pulse generation, where kisspeptin neurons are abundantly located, because kisspeptin antagonist abolishes the pulsatile release of gonadotrophs when injected to the arcuate nucleus, but not when injected into the POA in rats [26, 56]. Further, kisspeptin antagonist abolishes both GnRH pulses and basal discharge when injected into the median eminence (ME) of monkeys [50]. Pharmacological evidence revealed that injection of kisspeptin-10 and kisspeptin-54 in humans increased gonadotrophin pulsatile release, especially LH [57, 58]. The increase in pulsatile secretion of LH by kisspeptins has also been shown in human disease models [59-61]. It

has been suggested that kisspeptins also help in adjusting the hypothalamic clock of GnRH pulsatile release in addition to its stimulation in men [62], but the evidence does not support this effect in women [63]. The reason suggested for this was differential sex steroid feedback in females due to changes in hormonal levels across the menstrual cycle and sexual dimorphism in functional neuroanatomy of kisspeptins [1].

Kisspeptin neurons act as conduits for negative feedback of sex steroids

The suggestion that there must be some separate population of neurons that act as arbitrators to pass on the feedback signals from sex steroids to the GnRH neurons came from the fact that GnRH neurons do not express oestrogen receptors (ERs). Recent evidence suggests that KNDy neurons act as mediators to transmit both positive and negative signals from sex steroids to GnRH neurons [7]. The most striking feature of kisspeptin neurons is that almost all of them express oestrogen receptor alpha (ERα) and a major fraction also express oestrogen receptor beta (ERβ) [64, 65].

The initial evidence that kisspeptin neurons regulate sex steroids negative feedback came from studies done on rats, which revealed significantly increased expression of Kiss1 mRNA in the hypothalamus of both male and female rats after gonadectomy, with a parallel increase in levels of circulating gonadotrophins. Intriguingly, the above changes were reversed when these experimental models were replaced with sex steroids [66]. These findings were confirmed by similar studies using more refined techniques that also localised the changes to the arcuate nucleus [64, 65]. Further studies in different species including monkeys, pigs, sheep [67–69], and humans localised the same findings to the arcuate/infundibular nucleus after gonadectomy in animals and after menopause in humans [29]. Studies have also demonstrated that negative feedback effects of oestrogen are mainly mediated via ERα [64–66], which are expressed by kisspeptin neurons but not by GnRH neurons. This was proven by increased expression of Kiss1-mRNA associated with a marked increase in LH levels after selective elimination of ERα from mice models [70].

It has also been explained that in addition to ERα, the hypertrophied infundibular neurons in postmenopausal women also showed increased expression of neurokinin B-mRNA [71, 72], and evidence suggests that both neurokinin-B and kisspeptin function synergistically to deliver negative feedback of oestrogen [10, 40]. Similarly, neurokinin-B gene expression was increased in monkeys after ovariectomy, which was reduced by oestrogen supplementation [73].

The studies have also revealed dynorphin as a mediator of sex steroids negative feedback shown by increased expression of prodynorphin mRNA in the infundibular nucleus of postmenopausal women [74] and suppressed in arcuate nucleus of ovariectomised animals [11, 75–77]. These findings collectively suggest that sex steroids mediate their negative feedback to GnRH neurons by stimulating dynorphin secretion and suppressing neurokinin-B and kisspeptin secretion in the infundibular nucleus in humans.

Kisspeptin neurons act as conduits for positive feedback of sex steroids. Their role in preovulatory surge

Evidence from anatomical, expression, and pharmacological data supports the role of oestrogen and other sex steroids, such as progesterone, in moulding GnRH/LH responsiveness to kisspeptins, a fact that also contributes to the mechanisms for positive feedback and production of preovulatory surge. The evidence also elaborates that it is species and site specific [7].

Convincing evidence from studies performed on rodents suggest that oestrogen stimulates neurons at the AVPV (anteroventral periventricular nucleus), in association with activated progesterone receptors (PRs), to deliver its positive feedback by inducing preovulatory LH surge [7]. It was evident from decreased expression of Kiss1-mRNA at AVPV after gonadectomy, which increased after sex steroid replacement in both male and female mice [64, 65]. These findings were further confirmed by studies performed in rats [25, 78], which also revealed increased activation of kisspeptin neurons at AVPV (measured by increased c-fos expression) preceding oestrogen-induced preovulatory LH surges [78, 79]. Recent studies in rodents have also shown that kisspeptin neurons activation at AVPV follows an oestrogen-dependent circadian pattern [80–82], which receives its input from the suprachiasmatic nucleus (SCN) as evidenced by vasopressinergic (AVP) neuronal projections to kisspeptin neurons and the ability of AVP to augment their neuronal activity [80, 83].

In humans, anatomical evidence related to increased kisspeptin expression in response to oestrogens mainly comes from other species, which like humans have no area homologous to the AVPV nucleus, because no human studies have yet been done in this regard [1]. The evidence suggests that oestrogen increases the expression of kiss1-mRNA at the rostral periventricular nucleus in pigs [68] and POA/ARC in sheep during the late follicular phase corresponding to in vivo oestrogen peak preceding gonadotrophin surge [84–86]. There is also convincing evidence in humans suggesting mid-cycle surge of gonadotrophins by direct enhancement of

GnRH signalling at the pituitary instead of augmenting GnRH pulse secretion [87, 88].

The first pharmacological evidence favouring the role of sex steroids in shaping kisspeptin responsiveness came from rats that demonstrated that Kp-10 administration resulted in increased LH secretion with maximal release at the periovulatory period [89]. Recent pharmacological studies in animals have demonstrated that kisspeptin administration resulted in early LH surge [90] while preovulatory LH and FSH surge was attenuated by kisspeptin antagonist [91, 92]. In humans kisspeptin-54 injection generated LH surge and stimulated oocyte maturation with subsequent live term birth [93]. Similarly, another study in women showed kisspeptin-54 administration caused early LH surge and shortened menstrual cycle length [94]. In addition, injection of kisspeptin-10 in ovariectomised rats resulted in maximal gonadotrophin response only after oestrogen and progesterone replacement [89]. Further exploration revealed that selective blockade of ERα in rats resulted in significant suppression of LH response and reduced preovulatory LH surge to exogenous administration of Kp-10 (but no effects were observed on preovulatory LH surge after selective ERβ blockade) and enhanced the magnitude of acute response to Kp-10 [95, 96]. It suggested that oestrogen moulds gonadotrophin response to kisspeptins during the preovulatory period by maintaining a balance between ERα and ERβ signalling [96]. These studies also observed maximal response of gonadotrophin secretion when ovariectomised rats were supplemented with progesterone and selective ERα agonists favouring their potential role in inducing preovulatory gonadotrophins surge [7].

Kisspeptin effects on GnRH secretion in humans

Kisspeptin pharmacokinetics in humans

It is vital to understand the pharmacokinetics of kisspeptins for the proper interpretation of results of research studies administering kisspeptins in humans. The studies done in humans involving kisspeptin administration have used different kisspeptin isoforms (kp-10 and Kp-54), different techniques of administration (single or multiple boluses or continuous infusions), and distinct administration routes (subcutaneous [SC], intravenous [IV]) [97].

Both the isoforms of kisspeptins (Kp-10 and Kp-54) showed similar potency and activity when used on in vitro cultured cells [13, 14, 17], but studies in rodents demonstrated that Kp-10 had a slightly shorter onset and duration of action than Kp-54 even when both were given through same route and at same concentrations [47, 98]. The possible explanation for in vivo differences between the two isoforms is probably due to dissimilarities in their pharmacokinetic properties. While many studies have been done to explore the kisspeptin pharmacokinetics [99–101], the evidence is still not enough to understand the differences completely, although the available data is helpful to interpret the clinical studies being formed using different kisspeptin isoforms.

In short, it is comprehensible from the available evidence that Kp-54 decays slowly and has longer duration of action as compared to Kp-10, which decays very quickly. Secondly, studies have shown that more sustained levels of kisspeptin could be achieved after SC administration as compared to the IV route [97]. Evidence also suggests that kisspeptins are safe to administer as no adverse events, subject complains, changes in vitals, or changes in cell counts or liver and kidney function have been outlined by published reports [97].

Effects of acute kisspeptin administration in healthy subjects

Several studies have shown that kisspeptin strongly and directly stimulates GnRH and subsequently both LH and FSH secretion in humans, although the effect on LH secretion is much more pronounced [102]. Kisspeptin was first studied in healthy male volunteers, who received a 90-minute infusion of Kp-54 and showed significant and dose-dependent increase in plasma LH and less marked increase in FSH and testosterone [101]. Similarly, Kp-10 stimulated dose-dependent release of gonadotrophins after single IV bolus in both males and females [57, 100].

Studies in women have shown significant variation in response to kisspeptins across the menstrual cycle. A few studies have revealed a significant increase in LH secretion after a single IV bolus of Kp-10 in women, mainly in the preovulatory period [63, 100] when pituitary sensitivity to GnRH is usually enhanced due to positive feedback by oestrogen [97]. Inversely, markedly reduced responses were seen after Kp-10 injection or infusion in the early to mid-follicular phase, the phase in the menstrual cycle having lowest circulating oestrogen levels [100, 103], although some studies also found robust LH secretion during mid-luteal phase, which was not as significant as in the preovulatory phase [63]. Similarly, potent LH and FSH responses were observed mainly during the preovulatory period in women after sustained exposure to SC Kp-54 [99]. Intriguingly, a biphasic response was observed during the mid-luteal phase after SC Kp-54, which had been observed previously after long-acting GnRH analogues [104]. A possible explanation for this was differential pituitary behaviour to prolonged GnRH receptor acti-

vation. The studies also observed that the magnitude of gonadotrophins response to exogenous kisspeptins was greater than that to the endogenous kisspeptins, especially during the preovulatory and mid-luteal phases but not in the early follicular phase [63].

In brief, although the studies have used different kisspeptin isoforms, administration routes and doses, the results of all the studies are consistent in terms of kisspeptins effects on gonadotrophins secretion with more potent effects on LH release as compared to FSH. However, the response in women varies across the menstrual cycle, being markedly reduced in the early follicular phase, intermediate during the luteal phase, and immense in the preovulatory period.

Effects of kisspeptin administration in subjects with reproductive disorders

The effects of kisspeptin administration have also been studied in patients with reproductive disorders, having reduced gonadotrophin secretion. The first disease model studied was hypothalamic amenorrhea (HA), usually caused by stress, negative energy balance, and excessive exercise [105], and which is characterised by slow GnRH pulsatile secretion with subsequently low LH compared to FSH and reduced ovarian follicular activity. When Kp-54 was administered in these patients as a single SC bolus it resulted in significant elevations of LH and FSH, but there was no significant increase in oestrogen levels as indicated by ovarian inactivity on ultrasound scans [106]. Furthermore, when Kp-54 was given at an increased frequency (twice daily for two weeks) it resulted in an initial rise in LH levels, but after two weeks of treatment there was no detectable LH response. The reason suggested was kisspeptin receptor desensitisation with prolonged kisspeptin exposure [106, 107]. In order to avoid desensitisation, Kp-54 injections were given twice weekly for eight weeks. There was sustained gonadotrophins secretion over the eight weeks period although the response was reduced in the later period compared to day 1 and it again did not result in significant oestradiol release and ovulation was not achieved [107]. Furthermore, it has been shown recently that LH pulsatility could be achieved after Kp-54 infusion for eight hours with a three-fold rise in LH pulse frequency and mass per pulse [59], which supports the hypothesis that in order to restore reproductive endocrine activity in females with HA, pulsatile delivery of kisspeptins should be given because kisspeptin release is pulsatile [54, 108]. This is similar to the fact that in order to stimulate the reproductive axis GnRH must be delivered in a pulsatile mode [109].

The effects of exogenous kisspeptin have also been studied in men with type 2 diabetes having hypothalamic/pituitary hypogonadism with low testosterone. Such patients revealed robust increase in LH levels (two fold) after a single Kp-10 intravenous bolus and showed a more profound (five-fold) response after Kp-10 infusion for 11 hours. The results were comparable to the response seen in healthy males. In addition to the significant increase in LH levels, kisspeptin infusion also enhanced LH pulse frequency and raised testosterone levels to the physiological range in these patients. The levels were maintained over the course of infusion without any evidence of desensitisation as observed in females with HA. However, current evidence does not explain whether these effects will be maintained for longer periods in order to achieve therapeutic benefit, which needs further exploration.

One study looked at the effects of Kp-10 in patients with mutations in the neurokinin gene and its receptor (TAC3 and TACR3), which present with hypogonadotrophic hypogonadism [110]. These genes are thought to be involved directly or indirectly in modulating GnRH neuron secretion and the inability of neurokinin B to stimulate secretion of kisspeptin, and subsequent release of gonadotrophins is seen in these patients [111, 112]. When an infusion of Kp-10 was given to these patients, the number and the amplitude of LH pulses increased [61]. These observations revealed that GnRH neurons are intact in these patients, and kisspeptins do not need neurokinin B for their capability to stimulate GnRH secretion, which makes kisspeptins an appealing therapeutic approach to restore gonadotrophin secretion in patients with isolated GnRH deficiency [97]. It will also be helpful in future studies for further exploration in this aspect and prospective treatment for associated issues, such as fertility.

Sexual dimorphism to exogenous kisspeptin

Both men and women respond differently to exogenous kisspeptin, a phenomenon called sexual dimorphism. Kisspeptin administration stimulates significant gonadotrophin release in males, but the response is variable in women across the menstrual cycle. This phenomenon has been explored in both men and women using different kisspeptin isoforms, doses, and routes. The response observed in several trials on healthy male volunteers showed consistently that kisspeptins used in different isoforms, doses, and routes resulted in enhanced LH pulsatility and significant LH release [62, 101, 113]. However, the evidence related to females suggests that they respond differently to exogenous kisspeptins; one of the studies on healthy women did not show any detectable LH response in early follicular phase when Kp-10 was administered as an IV bolus, SC bolus, or IV infusion [100]. But a significant LH response was observed in another study in early follicular phase after using

a Kp-10 IV bolus. Similarly, remarkable LH response was seen in healthy women in early follicular phase after Kp-54 administration suggesting the response is better to longer isoform [99, 100]. The response observed was higher after Kp-10 administration in postmenopausal women and in women during the luteal phase as compared to women on sex steroid replacement and women in the early follicular phase of menstruation [99, 114]. These complex responses and relationships in females suggest that kisspeptin sensitivity is regulated across the menstrual cycle by changing levels of sex steroids and other possible unknown mechanisms. This sexual diversity in response to exogenous kisspeptins also explains the possible mechanisms that contribute to the generation of preovulatory surge of gonadotrophins, unique to females [1].

Continuous kisspeptin exposure leads to desensitisation

It is a remarkable feature of GnRH receptors that they undergo desensitisation after initial augmentation if they are stimulated by continuous GnRH administration [109, 115, 116], which has a therapeutic role for the treatment of prostate cancer [117]. Evidence from animal studies has shown that kisspeptins exhibit pulsatile secretion within the hypothalamus [108] and continuous Kp-10 stimulation leads to desensitisation of kisspeptin receptors as shown by an initial rise in LH levels followed by a rapid drop to baseline levels [118–121]. But when Kp-10 was given intermittently as twice daily injections it resulted in chronic stimulation of HPG-axis in both rats and monkeys [122, 123]. Moreover, reproductive function was recovered in rat models having HA by intermittent Kp-10 supplementation [124]. Similarly, in women with HA, desensitisation was observed after continuous SC administration of Kp-54 for two weeks [106], but the evidence is not consistent in other studies [1]. Recently in a study in women having HA, eight hours infusion of low dose Kp-54 restored pulsatile release of LH in addition to continuous LH secretion [59], and in another study in healthy women, their menstrual cycle was advanced by continuous administration of Kp-54 [94]. The studies done in healthy men showed varied results with no evidence of desensitisation to continuous infusion of Kp-10 at lower dose while LH response was reduced when continuous infusion of high dose Kp-10 was given for 24 hours [1, 57].

These observations therefore suggest that Kp-10 and Kp-54 desensitise the kisspeptin receptors at higher dose but not at lower dose, as further clarified in a dose-finding study. The alternative reason suggested was that kisspeptins at high dose might stimulate gonadotrophin inhibitory receptor to reduce any further rise in GnRH and LH [1, 57]. This feature of kisspeptin receptors has a therapeutic potential, and two kisspeptin receptor agonists (TAK-448 and TAK-683) have already been developed, which have shown promising results in recent phase I clinical trials in healthy men for future use in suppressing gonadotrophin levels, similar to GnRH analogues [125, 126].

The role of kisspeptins in puberty onset

The initial indication for a crucial role of kisspeptins in puberty came from studies in humans and mice, which revealed that inactivating mutations of GPR54 were associated with impaired pubertal development [3, 5, 18, 19]. Subsequently, activating mutations of kisspeptin receptor gene were found in patients with precocious puberty [127, 128]. These findings attracted substantial attention and have been the subject of rigorous analysis in various mammalian and non-mammalian species [92, 129] and the current available neuroanatomical and functional evidence, although mainly in rodents, strongly supports the role of kisspeptins in triggering the onset of puberty [129, 130].

Studies in primates and rodents have shown increased hypothalamic expression of kiss1 gene and kiss1r mRNA during pubertal development [66, 131]. Pharmacological studies revealed that repeated administration of Kp-10 resulted in advanced pubertal development in immature rats and enhanced GnRH secretion in juvenile monkeys [122, 123]. Further, studies in rodents have shown that during puberty there was an increased quantity of GPR54 expressing GnRH neurons, increased GPR54 signalling efficiency, and enhanced sensitivity to kisspeptin stimulatory effects indicated by increased gonadotrophin secretory response during puberty [31, 39, 132]. There was also less propensity of GPR54 to desensitisation to continuous stimulatory effects of kisspeptins during pubertal development in female rats [133]. Recent immunohistochemical data showed a sharp rise in the number of kisspeptin neurons and kisspeptin neuron projections to the GnRH neurons at onset of puberty in female rodents involving oestrogen stimulating signals, suggesting that early stages of ovarian activation occur before complete stimulation of kisspeptin neurons during puberty, which serve as an amplifier for gonadotrophin secretion along pubertal maturation [134]. Conversely, kisspeptin antagonist administration attenuated pulsatile release of gonadotrophins in monkeys during puberty and slowed pubertal growth in rats [50, 92].

Collectively, all of these findings suggest that kisspeptin signalling has an indispensible role in triggering complex activational mechanisms required for initiating puberty in a range of species.

The role of kisspeptins in metabolic control of puberty and reproduction

It has been observed that pubertal growth and reproduction in humans and other species is affected by both extremes of nutritional behaviour, including malnutrition and over-nutrition (and obesity). Current evidence suggests that kisspeptins function downstream to nutritional and metabolic signals; they transmit information related to energy stores to gonadotrophin neurons and serve as a connection between reproduction and the metabolic status of the body. Evidence related to the role of kisspeptins in metabolic control of reproduction comes from studies done in both animals and humans. Studies in rodents and primates have shown reduced gonadotrophin secretion along with decreased expression of Kiss1 mRNA after fasting [124, 135–137], and kisspeptin administration in rats has been shown to restore delayed pubertal features associated with malnutrition [122, 124].

The data also suggests that leptin affects the timing of puberty by regulating kisspeptin neurons. Leptin deficiency has been found to be associated with hypogonadotrophic hypogonadism and impaired pubertal development in humans [138, 139], and leptin administration resulted in normal pubertal development in both male and female patients [140]. The idea that kisspeptins are involved in mediating leptin signals to the HPG axis came from the finding that leptin receptors are not present on GnRH neurons but on kisspeptin neurons. Kiss1 mRNA expression was reduced in leptin-deficient mice, which was enhanced by leptin administration [141], but evidence also suggests that leptin signalling is not obligatory for reproduction and normal pubertal development [142].

The reason for hypogonadotrophic hypogonadism with reduced testosterone levels found in obese patients with type 2 diabetes mellitus [143] has been suggested due to reduced kisspeptin stimulatory effects [144]. It was proven by a study in the rat model of diabetes in which reduced levels of hypothalamic Kiss1 mRNA and gonadotrophins were restored by kisspeptin administration [145, 146]. Similarly, in humans reduced gonadotrophin levels in patients with obesity and type 2 diabetes mellitus were restored by kisspeptin administration [60]. This gives hope for future therapeutic potential in restoring reproductive function in patients suffering from conditions of negative energy balance, like diabetes and anorexia nervosa.

Kisspeptin actions on the reproductive system in addition to the hypothalamus

Although current data suggests that the hypothalamus is the primary site of action of kisspeptins, convincing evidence also suggests that kisspeptins act on other levels of the reproductive system.

Pituitary

The first evidence related to the direct action of kisspeptins at the pituitary came from studies done on rats, which showed increased LH secretion from pituitary in vitro [52, 147]. Later studies in many other species showed the same results, where kisspeptin was able to stimulate LH secretion directly from pituitary cells [53, 54, 148–151]. Likewise, expression studies in rats and monkeys have shown that both kiss1 and GPR54 mRNAs are expressed at pituitary cells that are hormonally regulated by oestrogen and GnRH [51, 53]. Additionally, kisspeptins have been found in hypophyseal portal blood of sheep [54]. Collectively, the above-mentioned data significantly suggest the role of kisspeptins in direct pituitary stimulation to release gonadotrophins at certain times, but it is essential to mention that some other studies in rats have been unable to find any kisspeptin action on pituitary [46, 152]. Overall, these negative results do not disprove the likelihood of direct pituitary action of kisspeptin in controlling the gonadotrophic axis, which nevertheless demands further validation.

Female reproductive tract

It has been suggested that kisspeptins modulate follicular maturation, oocyte survival, and subsequent ovulation. The initial evidence regarding the presence of kisspeptin and its receptor on the ovaries and uterus came from studies done on rats [153], which was later confirmed by immunohistochemical analysis performed in other species including humans [154–158]. Interestingly, in these studies it was observed that kiss1 mRNA expression occurs in a cyclic manner during the oestrous cycle with a significant increase at the preovulatory stage, which could be prevented by attenuating preovulatory LH surge and could be restored by hCG treatment [154]. Similar results were observed in indomethacin-treated rats and photo-inhibited hamsters [155, 157]. Additionally, recent studies on kiss1r heterozygous and knockout mice models further provide strong evidence in favour of the direct role of kisspeptins in the ovary by revealing that the loss of one kiss1r allele resulted in premature ovarian failure and loss of both kiss1r alleles blocked maturation of ovarian follicles and ovulation, which could not be rescued by gonadotrophins administration [159–161]. It has also been suggested that localised kisspeptin signalling is crucial for endometrial decidualisation and embryo implantation. It has been shown by studies in rats [160] that showed impaired embryo implan-

tation in kiss1 knocked out mice that could not be rescued by gonadotrophins administration indicting the defect is uterine based. It was explained further that localised uterine kisspeptin signalling modulates embryo implantation by regulating localised leukocyte inhibitory factor (LIF) secretion, which has been shown to be critical for implantation in mice [162, 163]. Similarly, studies in mice have shown increased expression of kiss1 and kiss1r at the time of endometrial decidualisation, and the process was attenuated by downregulating kiss1 expression [164].

Kisspeptins have been suggested to play a critical physiological role in human placental function and implantation by modulating invasion of placental trophoblast cells into the endometrium. It has been demonstrated by the presence of high levels of Kiss1 gene and kisspeptin in the human placenta and their role in controlling human extravillous trophoblasts' (EVTs) migratory and invasive properties [14, 17, 163, 165–167]. Recently it has also been revealed that kisspeptin and its receptor play an important role in regulating placental angiogenesis, a critical process in successful establishment of placenta, required for normal foetal growth and development and for maintenance of a healthy pregnancy [168–171]. Furthermore, high levels of kisspeptins have been found in pregnant women as compared to non-pregnant women, suggesting kisspeptin is placenta derived [167]. The levels rise substantially as the pregnancy progresses and stay high until parturition [167], which has been suggested to play an important role in negatively modulating the trophoblastic invasion in later pregnancy [106, 172–174]. Additionally, low levels of kisspeptins have been found to be associated with gestational diabetes mellitus as they stimulate glucose-dependent insulin secretion [175, 176].

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 [177]. 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 [178, 179].

Clinical utility of kisspeptins in reproduction

On the basis of pharmacological and physiological data as discussed above, 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 insufficiency of central origin provided the GnRH neuronal system is intact. Exogenous kisspeptin administration has been shown to initiate puberty in rodents and monkeys [122, 123], and restored pulsatile secretion of LH in diabetic men having central hypogonadism, and in women with hypothalamic amenorrhea [60, 107]. Similarly, kisspeptin restored pulsatile gonadotrophin secretion in patients having central hypogonadism due to NKB or its receptor mutations [61], although NKB administration in healthy males or females did not cause any significant alterations in reproductive hormone secretion [180]. Kisspeptin has also been revealed recently to induce oocyte maturation in women with subfertility undergoing IVF (in vitro fertilisation) treatment with successful attainment of live birth [93, 181]. 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 [93]. Also, the ability of different kisspeptin forms (Kp-54, Kp-10) to strongly stimulate secretion of gonadotrophins in humans suggest the development of optimal protocols (dose, duration, and pattern) of kisspeptin administration to activate the HPG axis by kisspeptin analogues without the development of gonadotrophic system desensitisation [57, 62, 99, 101, 106, 113].

Conversely, kisspeptin antagonists have been clearly shown to diminish the frequency and amplitude of LH pulsatile release without affecting basal LH secretion [50], which can be helpful in situations of increased gonadotrophins pulsatility where a diminished rather than complete suppression is required. Secondly, in contrast to GnRH analogues, kisspeptin antagonists cause less profound reduction in LH pulsatility, with consequently fewer chances of side effects associated with GnRH analogues, including loss of libido, hot flushes, and reduced bone mineral density [1, 50]. Conditions such as endometriosis, uterine fibroids, and benign prostatic hyperplasia might benefit from kisspeptin antagonists, where limited suppression of gonadotrophins could improve the pathologies without having side effects of complete suppression associated with GnRH analogues [7]. Similarly, the ability of kisspeptin antagonists to impede LH ovulatory surge without affecting its basal levels offers a potentially novel female contraceptive in which ovulation would be suppressed but oestrogen production and follicular development would continue [92]. 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) [1, 182]. In a recent randomised trial researchers 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 [183]. Likewise, kisspeptin and NKB antagonists might be helpful in treating patients having precocious puberty [1] with the additional benefit of reduced menopausal side effects [184-186].

Conclusions

There is robust evidence available in favour of kisspeptins as a central modulator of pulsatile gonadotrophin secretion, which plays a pivotal role in controlling the onset of puberty and reproduction in both human sexes. There is also significant evidence available suggesting kisspeptins mediate both positive and negative sex steroid feedback signals to GnRH neurons and serve as a vital connection between the reproduction and metabolic status of the body. Exogenous kisspeptin administration in humans has also been recognised as a potential novel therapeutic approach for treating disorders with either pathologically reduced or augmented gonadotrophin pulsatile secretion and is currently a focus of translational research. The studies in humans have used different kisspeptin isoforms, administration routes, and doses, but the results are consistent in terms of the effect of kisspeptins on gonadotrophin secretion. A great deal of work has been done in this regard, but there is still a need to develop appropriate protocols for kisspeptin administration before they can be used for infertility and reproductive disorders in humans. Kisspeptins have also been identified in several peripheral reproductive organs indicating their role in modulating vital physiological processes, including ovarian function, embryo implantation, and placentation, but more robust evidence is required before making these findings relevant in humans because current evidence is only available from studies done on animal models.

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