

Clinical Translational Studies of Kisspeptin and Neurokinin B

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Abstract

Kisspeptin and neurokinin B (NKB) are hypothalamic neuropeptides that are vital for reproductive health. An absence of either kisspeptin or NKB signaling results in hypogonadotropic hypogonadism and a failure to proceed through puberty. In recent years, several studies have demonstrated potential avenues for the clinical utility of medications that act through these pathways in the assessment and treatment of reproductive disorders. Kisspeptin acts to stimulate hypothalamic gonadotrophic-releasing hormone (GnRH) secretion from the hypothalamus. Kisspeptin induces gonadotrophin secretion in both healthy men and women, and in women with reproductive disorders such as hypothalamic amenorrhea (HA). Kisspeptin-based treatments hold promise for use during in vitro fertilization (IVF) treatment; a bolus of kisspeptin-54 induces an LH surge of 12 to 14 hours of duration sufficient to induce oocyte maturation, but with markedly reduced rates of the most significant complication of IVF treatment, ovarian hyperstimulation syndrome (OHSS). Kisspeptin could also be used chronically to restore reproductive health in patients with functional hypogonadism, such as those with HA. Furthermore, kisspeptin has potential as a diagnostic test of hypothalamic function; a “kisspeptin test” could be used in children with delayed puberty to identify the subset with genetically determined deficits in hypothalamic pathways (congenital hypogonadotropic hypogonadism [CHH]). In addition to its role in hypothalamic GnRH pulse generation, NKB plays a critical role in the occurrence of one of the most troubling symptoms of the menopause, the “hot flush.” Neurokinin-3 receptor (NK3R) antagonists are highly effective as treatments for hot flushes in postmenopausal women, with several compounds now in late-phase development. Furthermore, NK3R antagonism leads to a reduction in LH secretion by reducing GnRH pulsatility in the hypothalamus and has been shown to reduce androgen levels in women with polycystic ovary syndrome (PCOS) (in whom GnRH pulsatility is often increased). In summary, although further detailed evaluation in several clinical settings is ongoing, medications based on kisspeptin and NKB pathways have prodigious potential in the assessment and treatment of reproductive disorders.

Keywords

- ▶ Kisspeptin
- ▶ neurokinin B
- ▶ reproductive disorders

Kisspeptin and neurokinin B (NKB) are hypothalamic neuropeptides that are obligate for reproductive health. In 2003, two seminal publications reported that inactivating mutations in the gene encoding for the kisspeptin receptor (previously known as GPR54) cause hypogonadotropic hypogonadism

and a failure of pubertal development.^{1,2} Subsequently, it was found that an inactivating mutation of the *KISS1* gene also results in normosmic hypogonadotropic hypogonadism.³ Conversely, activating mutations of the kisspeptin receptor result in central precocious puberty.⁴ Thus, kisspeptin was

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shown to play a key role in orchestrating reproductive hormonal secretion and in the regulation of normal puberty.

Similarly, mutations in genes encoding for NKB or its receptor (TAC3R) also result in congenital hypogonadotropic hypogonadism (CHH). The decapeptide NKB is encoded for by the TAC3 gene and activates the neurokinin 3 receptor (NK3R) encoded by the TAC3R gene.^{5–9} Patients with NK3R mutations have reduced gonadotrophin-releasing hormone (GnRH) pulsatility with an increase in follicle-stimulating hormone (FSH) to luteinizing hormone (LH) ratio.¹⁰ Patients with either TAC3 or TAC3R mutations were shown to remain responsive to exogenous kisspeptin administration, consistent with NKB exerting its action upstream of kisspeptin.^{10,11}

Since then, it has been established that kisspeptin acts to stimulate hypothalamic GnRH neurones and thus the remainder of the reproductive axis. In rodent models, kisspeptin was found to co-localize with NKB and dynorphin to form “KNDy” neurones. These neuropeptides are thought to act in a paracrine manner to result in the pulsatile secretion of GnRH. Both central and peripheral administration of kisspeptin in animal models results in robust stimulation of gonadotrophin secretion.^{12,13}

Kisspeptin in Healthy Men and Women

In 2005, Dhillon and colleagues conducted the first administration of kisspeptin to healthy men and observed a dose-dependent increase in gonadotrophin secretion.¹⁴ Thereafter, the same group administered kisspeptin to healthy women; while significant increases in gonadotrophins were observed in the follicular and luteal phases, LH-rise was most prominent during the preovulatory phase of the menstrual cycle.¹⁵ Similarly, Chan et al observed that women were least sensitive to an intravenous bolus of kisspeptin-10 during the follicular phase of the menstrual cycle.¹⁶

A key feature of a healthy reproductive axis is the pulsatile secretion of GnRH [by proxy serum LH]. A single subcutaneous bolus of kisspeptin was shown to increase LH pulsatility in healthy women during the follicular phase.¹⁷ Furthermore, a single bolus of kisspeptin-10 could reset the GnRH pulse generator in men,¹⁸ but this did not appear to be the case in women.¹⁶

Acute LH responses to intravenous bolus administration of kisspeptin-10 were investigated in healthy men (dose range: 0.01–3 µg/kg) and the highest LH rises were observed after 1 µg (0.77 nmol/kg).¹⁹ Moreover, a continuous intravenous infusion of 4 µg/kg per hour (3.07 nmol/kg per hour) of kisspeptin-10 persistently stimulated LH secretion over 22.5 hours.¹⁹ Intriguingly, the LH response to an acute intravenous bolus of kisspeptin-10 (0.3 µg/kg) was similar in obese hypogonadal diabetic men to that in healthy control subjects.²⁰ Furthermore, robust LH stimulation was observed to an intravenous infusion of kisspeptin-10 (4 µg/kg per hour) in obese hypogonadal diabetic men for 11 hours.²⁰ Additionally, responses to kisspeptin in healthy older men were also maintained.²¹ Thus, in future, kisspeptin-based therapeutics could be developed for the treatment of functional hypogonadism such as diabetes, obesity, or age-related hypogonadism.

Kisspeptin in Hypothalamic Amenorrhea

Hypothalamic amenorrhea (HA) is a condition characterized by a reduction in the physiological pulsatile secretion of GnRH. HA frequently occurs in the context of low body weight, excessive exercise, reduced energy availability, psychological stress, or genetic predisposition.²²

Notably, the acute LH response to kisspeptin was increased by four-fold in women with HA compared with the same dose in healthy women during the follicular phase.²³ Data from a rodent model of HA could explain this observation; *Kiss1* expression in the hypothalamus is reduced in undernourished rodents, whereas kisspeptin receptor expression is increased.²⁴ Thus, HA could be considered as a state of hypothalamic kisspeptin deficiency, and thus there has been great interest in investigating the use of kisspeptin to restore physiological hormonal secretion in these women. Twice daily subcutaneous administration of kisspeptin-54 (6.4 nmol/kg) for 2 weeks was investigated in five women with HA.²³ While robust increases in serum gonadotrophin levels were observed on day 1 of administration, these were reduced within a few days of administration,²⁵ and markedly attenuated by day 14.²³ The majority of the tachyphylaxis to kisspeptin is likely to have occurred at the level of the kisspeptin receptor as GnRH responsiveness was maintained.²³ However, twice weekly administration of subcutaneous kisspeptin was able to persistently stimulate gonadotrophin secretion.²⁵ Thus, whilst bolus administration of kisspeptin has potential to restore normal reproductive function in women with HA, there is a risk of tachyphylaxis with excessive doses of kisspeptin. An alternative approach is to use a continuous intravenous infusion of kisspeptin, which when administered at lower doses can restore physiological LH pulsatility without tachyphylaxis.²⁶ Ongoing studies are assessing whether continuous subcutaneous administration of kisspeptin could be used to restore ovulatory function in women with HA.

Kisspeptin During *In Vitro* Fertilization

Kisspeptin signaling is requisite for physiological ovulation; infusion of a kisspeptin neutralizing antibody directly into the preoptic area of the hypothalamus results in abolition of the midcycle LH surge.²⁷ Furthermore, kisspeptin administration to superovulated prepubertal rats was able to induce ovulation to a similar extent as human chorionic gonadotrophin (hCG).¹² Additionally, the LH response to kisspeptin is dramatically increased in the preovulatory phase of the menstrual cycle, suggesting that it could induce an ovulatory LH surge.¹⁵

Indeed in 2014, Jayasena and colleagues conducted a “proof of concept” study demonstrating that a single subcutaneous bolus of kisspeptin-54 (1.6–12.8 nmol/kg) was able to induce an LH surge with a peak of approximately 40 IU/L that lasted for 12 to 14 hours.²⁸ This was sufficient to induce oocyte maturation in 51 of 53 healthy subfertile women undergoing *in vitro* fertilization (IVF) treatment.²⁸ Furthermore, kisspeptin safely induced high rates of oocyte maturation in 60 women at increased risk of ovarian hyperstimulation syndrome (OHSS)

based on elevated ovarian reserve markers without causing this most serious of complications of current IVF treatment.²⁹ OHSS is predominantly caused by hCG stimulating the release of vascular endothelial growth factor (VEGF) from the ovary, which increases vascular permeability and leakage of fluid into the third spaces of the body, resulting in ascites, pleural effusions, renal impairment, and rarely even death.³⁰ Kisspeptin reduced the odds of severe OHSS by 33.6-fold (95% CI: 12.6–89.5) compared with hCG.³¹ More recently, kisspeptin has been hypothesized to play a direct role in the pathogenesis of OHSS, by directly reducing estradiol-induced VEGF production.³² This is consistent with clinical data, suggesting that extending the duration of the LH surge with a second dose of kisspeptin further enhances oocyte maturation, but without causing OHSS.³³

Kisspeptin is known to be present in the ovary and has been suggested to play a direct ovarian role in addition to its predominant mode of action through hypothalamic GnRH secretion. Ovarian kisspeptin expression is undetectable in immature oocytes, but is increased at ovulation.³⁴ Kisspeptin has been shown to increase *in vitro* maturation of ovine³⁵ and porcine immature oocytes,³⁶ and smaller follicles contributed more to the oocyte yield than following other triggers in humans.³⁷ Furthermore, intrafollicular kisspeptin levels correlate with the number of mature oocytes retrieved.⁵

Owens and colleagues³⁸ examined the *in vivo* and *in vitro* actions of using kisspeptin-54 trigger on gene expression relating to ovarian reproductive function, steroidogenesis, and OHSS in granulosa lutein cells, compared with current triggers.³⁸ They observed that triggering oocyte maturation with kisspeptin-54 increased the expression of genes involved in ovarian steroidogenesis such as LH/hCG and FSH receptor, steroid acute regulatory (STAR) protein, aromatase, and estrogen receptors.³⁸

In summary, kisspeptin stimulates release of an LH surge sufficient to induce effective oocyte maturation and achieve at least comparable pregnancy rates in subfertile women undergoing IVF treatment. Crucially, rates of OHSS are dramatically reduced by kisspeptin triggering, and this could in part be achieved by a further direct ovarian action of kisspeptin on VEGF production. Randomized controlled trials directly comparing kisspeptin to current triggers of oocyte maturation with accurate determination of clinical outcomes, such as OHSS rates, are now indicated.

Kisspeptin as a Diagnostic Test of Hypothalamic Function

Kisspeptin acts to stimulate the hypothalamus to secrete GnRH and thus has potential as a diagnostic test of hypothalamic reproductive function, where currently no direct test exists. The hypothalamus is known to play a key role in controlling the onset of puberty; mutations in several genes involved in the regulation of hypothalamic GnRH function are known to result in CHH and absent puberty. Responses to kisspeptin in CHH have been shown to be attenuated³⁹; however, patients with reversal of CHH (which can occur in up to one-fifth of such patients) regain responsiveness to

kisspeptin.⁴⁰ The majority of patients with delayed puberty have constitutional delay of growth and puberty (CDGP) and will proceed through puberty with time; however, a subset of patients will have CHH and are unlikely to proceed through puberty without treatment. Thus, kisspeptin has been evaluated as a diagnostic test in children with delayed puberty and a wide variety of responses to a kisspeptin challenge test were observed.⁴¹ Follow-up of puberty onset in these children will reveal whether those who responded to kisspeptin were more likely to have CDGP than CHH.

In summary, mutations affecting structural components of hypothalamic GnRH signaling (e.g., anosmin1) are unlikely to respond to kisspeptin (unless reversal has occurred)⁴⁰; however, functional hypogonadotropic hypogonadism (e.g., HA²³ or hyperprolactinemia⁴²) does respond to kisspeptin. Thus, a “kisspeptin test” can be used to more accurately evaluate the hypothalamic contribution to hypogonadotropic hypogonadism.

NKB Administration in Humans

NKB administration in male agonadal juvenile monkeys increases LH levels.⁴³ However, infusions of NKB were unable to stimulate gonadotrophin secretion or pulsatility in either healthy men or women.⁴⁴ Nevertheless, an interesting phenomenon was reported by some participants who had received higher doses, in that they felt hot and appeared flushed.⁴⁴ This was investigated in more detail in 10 healthy women who received a 30-minute infusion of either NKB or vehicle in random crossover design.⁴⁵ Sweating, heart rate, and skin temperature were increased during NKB administration, which are characteristic features of menopausal flushing.⁴⁵ Following this, NK3R antagonists have been developed as a novel therapy for menopausal flushing, reducing the total number and severity of flushes by 41 to 45%.⁴⁶ Thus, these promising agents could revolutionize the treatment of women with hot flushes, especially those at risk of side effects from sex-steroid-based therapies, and several compounds are now in late-phase development.

NK3 Receptor Antagonism in Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a condition characterized by anovulation, hyperandrogenism, and polycystic ovarian morphology.⁴⁷ Increased GnRH pulsatility is implicated in the pathogenesis of PCOS and is often reflected by an increase in serum LH level, which is estimated to be present in half of women with PCOS. Thus, kisspeptin and NKB pathways could be targeted to treat women with PCOS. George and colleagues investigated the use of pharmacological blockade of NKB action to reduce GnRH pulsatility, and assessed the impact of this on clinical features of PCOS such as hyperandrogenism.⁴⁸ Women were randomized to receive an oral NK3R antagonist (AZD4901 at a dose of 20, 40, or 80 mg/day) or placebo for 28 days.⁴⁸ After 7 days of 80 mg/day, a 52% reduction in area under the curve (AUC) of LH levels and a reduction in LH pulsatility by 3.6 LH pulses

per 8 hours ($p < 0.05$) was observed.⁴⁸ This was associated with a reduction in total testosterone levels by 28.7%.⁴⁸ A secondary analysis revealed that this reduction in androgen levels predominantly occurred in anovulatory women.⁴⁸ Further longer-term studies are indicated to evaluate the efficacy of this novel approach in the treatment of ovulatory dysfunction in women with PCOS.

Conclusion

Kisspeptin is a critical regulator of hypothalamic GnRH function, and thus offers a significant opportunity to better evaluate and treat conditions caused by hypothalamic dysfunction. Several kisspeptin analogs are in development,^{49–54} which can aid in the translation of kisspeptin-based therapeutics to the bedside. Medications targeting NKB signaling are likely to represent a huge advance in the management of women with postmenopausal hot flashes and could have benefit in other conditions such as PCOS.

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Conflict of Interest

There are no conflicts of interest to declare.

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