Kisspeptin and the Hypothalamic Control of Reproduction: Lessons from the Human

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Abstract

The hypothalamic hormone GnRH is a central driver of pituitary gonadotropin secretion, controlling pulsatile gonadotropin secretion, modulating gonadal steroid feedback, and bringing about full fertility in the adult. Thus, understanding GnRH neuronal regulation is essential to understanding the neurohumoral control of human reproduction. Genetic tools were used in patients with GnRH deficiency (i.e. idiopathic hypogonadotropic hypogonadism), a clinical syndrome that results from the failure of a normal pattern of pulsatile GnRH, to discover upstream modulators of GnRH secretion (1). In 2003, homozygosity mapping of two consanguineous pedigrees led to the identification of loss of function mutations in KISS1R (a G protein coupled receptor) by two groups (2, 3). In parallel, the Kiss1r−/− mouse was shown to be a phenocopy of the human GnRH-deficient state, demonstrating that the function of KISS1R/Kiss1r is conserved across mammalian species (4). Just before these human genetic discoveries, the ligand for kisspeptin-1 receptor [KISS1R; also known as G protein coupled receptor 54 (GPR54)], was discovered to be kisspeptin. Soon thereafter a large array of experimental studies began assembling genetic, expression, physiologic, transgenic, knockdown, and electrophysiological data to characterize the physiology of kisspeptin and its seminal role in modulating GnRH release.

Kisspeptin is now recognized as critical regulator of the timing of sexual maturation, the sexual differentiation of the brain, the adult regulation of gonadotropin secretion by gonadal hormones, and the control of fertility by metabolic and environmental (e.g. photoperiod) cues (5, 6). Although most of the in vivo data have been obtained in rodent and nonhuman primate species, kisspeptin has also been used as a physiological probe in human investigation. In just a short time, these studies have contributed greatly to our understanding of the neuroendocrine mechanisms responsible for GnRH induced gonadotropin secretion in the human, in both normal and pathophysiological states.

KNDy network

Kisspeptin is now appreciated to be coexpressed with other neuropeptides that are likely to work in a cooperative
fashion to regulate the hypothalamic control of reproduction. Kisspeptin neurons in the arcuate nucleus coexpress the neuropeptides neurokinin B (NKB) and dynorphin, giving rise to the term KNDy neurons (kisspeptin-neurokinin B-dynorphin); this colocalization has been observed in several mammalian species including humans (7–9). NKB is a member of the substance P-related tachykinin family and its receptor is expressed both on KNDy and GnRH neurons (10). Dynorphin is an opioid that participates in progesterone-mediated negative feedback control of GnRH release (11, 12). Just as loss-of-function mutations in KISS1R (kisspeptin-1 receptor) (2, 3) and KISS1 (13) were identified in patients with GnRH deficiency, loss-of-function mutations in the genes encoding neurokinin B (TAC3) and its receptor (TAC3R) result in normosmic hypogonadotrophic hypogonadism and pubertal failure (14). Although mutations in the genes encoding dynorphin or its receptor have not yet been reported, the presence of mutations in the other two signaling pathways in GnRH deficient states demonstrates the importance of this neuropeptide network in the hypothalamic control of reproduction. Thus, kisspeptin is likely not to act alone but is part of a complex hypothalamic neuropeptide network that in aggregate works to modulate GnRH release.

Translating kisspeptin physiology from animals to the human

Physiological studies of kisspeptin's actions have been performed in several animal species. Both central (intracerebroventricular) and peripheral administration of kisspeptin result in a marked rise in plasma LH and to a lesser extent FSH in several mammalian species including mice (15, 16), rats (17, 18), sheep (19), and monkeys (20). Because pretreatment with a GnRH antagonist abolishes kisspeptin's stimulatory effect, the effect of kisspeptin on gonadotrophin release is likely to be due to kisspeptin stimulation of GnRH release into the portal circulation, which in turn stimulates the release of LH and FSH from the gonadotrophs of the anterior pituitary gland (16). In fact, the majority of hypothalamic GnRH neurons express GPR54 (G protein coupled receptor 54) (18, 19, 21). Moreover, kisspeptin administration has been shown to induce the release of GnRH from rat hypothalamic explants (15, 22), to increase electrical activity (21), and to induce c-fos immunoreactivity in rodent hypothalamic GnRH neurons (17, 21). Central injection of kisspeptin-10 to sheep is followed by a rise in cerebrospinal fluid GnRH levels, which parallels the observed rise in serum LH (19). Although Kiss1 neurons clearly project directly to GnRH neurons, not all of its actions may be direct because there is evidence that kisspeptin may also act indirectly through other neurotransmitters to modulate GnRH release (23, 24).

Again, the belief that the primary site of action of kisspeptin is the GnRH neuronal population stems, in part, from the observation that administration of GnRH antagonists before kisspeptin administration blunts the stimulatory effects of kisspeptin (16, 20). To date, there have yet to be human studies in which kisspeptin administration is preceded by administration by a GnRH antagonist or long-acting analog. Nonetheless, due to the consistency of the physiological observations in mice and nonhuman primates, it is presumed that the primary mechanism of kisspeptin's actions is GnRH neuronal activation. Thus, the ability to administer kisspeptin to humans is a seminal step in unraveling the mechanisms linking the complex kisspeptin-neurokinin B-dynorphin neuropeptide network. Because GnRH is difficult to measure in peripheral blood and LH levels are traditionally used as a surrogate marker of GnRH secretion, measuring the changes in serum LH levels after kisspeptin is therefore a means to understanding the triggers for GnRH secretion, the duration of GnRH secretory activity, and the oscillatory properties underlying GnRH neurons. Therefore, although kisspeptin has the ability to result in marked elevations of LH in the peripheral blood (see below), its power as a physiological probe lies in its ability to teach us about the fundamental secretory properties of GnRH neurons in vivo.

Sex steroid context

In the human, kisspeptin administration has occurred in subjects with intact hypothalamic-pituitary-gonadal axes and well as subjects with reproductive diseases characterized by low sex steroid levels. Sex steroids play pivotal
roles in the control of GnRH and gonadotropin secretion because it has been appreciated for decades that estrogen can stimulate gonadotropin release at certain times within the ovarian cycle (positive feedback) and suppress it at others (negative feedback). Because both positive and negative feedback have thought to be mediated via estrogen receptor-α neuronal signaling (25), the absence of such receptors on GnRH neurons have long suggested that other pathways (i.e. kisspeptin) can play a role in this process. Indeed, sex steroids have been shown to have profound effects on the transcriptional regulation of Kiss1 gene expression in the rodent, with estrogen up-regulating the expression of Kiss1 at the anteroventral periventricular nucleus and down-regulating it at the arcuate nucleus (26, 27). Moreover, sex steroids are able to modulate the GnRH responsiveness to kisspeptin. For example, kisspeptin has been shown to increase γ-aminobutyric acid and glutamate transmission to GnRH neurons in an estradiol-dependent manner in the mouse (23). In addition, blockade of estrogen receptor-α in female rats reduces the acute gonadotropin response to kisspeptin (28, 29). Therefore, the sex steroid milieu of the human is likely to influence the degree of GnRH and/or gonadotropin responsiveness to kisspeptin.

The number of articles now published in the literature regarding the administration of kisspeptin to both healthy volunteers and patients with reproductive disorders (30–36) is still relatively small, but it can be difficult to compare studies directly due to the different isoforms of kisspeptin that have been used [kisspeptin 68–121 (54-mer), kisspeptin 112–121 (decapeptide)], methods of administration (iv, sc), types of exposure (single bolus, continuous), chronicity of administration (single bolus, multiple doses), and most importantly, study populations (healthy volunteers, patients with reproductive disorders) that have all been used. However, in aggregate, several concepts emerging from these important studies are providing new insights into the secretory properties of GnRH neurons and thus are worthy of review.

**Effect of kisspeptin administration on GnRH-induced gonadotropin secretion in healthy men**

Exogenous kisspeptin stimulates the secretion of both gonadotropins in men. This is true whether kisspeptin is given as a brief infusion (31) or as a single bolus (30, 35), but the effect on LH secretion appears to be more pronounced than FSH (31). Kisspeptin results in a rapid and dose-dependent rise in LH (31), although in one study administration of the highest dose of kisspeptin (3 µg/kg, iv) elicited a smaller LH response than a lower dose (1 µg/kg, iv), raising the possibility that kisspeptin can bring about rapid hypothalamic desensitization of its own receptor (30). Kisspeptin-induced, GnRH-induced LH pulses are grossly similar to endogenous LH pulses; however, the LH pulses induced by kisspeptin are more rounded and prolonged, with a longer time from nadir to peak (35). It has been determined that the shape of LH pulses produced by kisspeptin would be mimicked by a 17-min infusion of GnRH (35), a duration that is strikingly concordant to kisspeptin-induced GnRH neuronal firing *ex vivo* (21, 37–40). **Table 1** summarizes the LH secretory profile observed in healthy eugonadal men.

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<th><strong>Table 1.</strong> Responses to intravenous kisspeptin boluses in healthy men</th>
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**Effect of kisspeptin administration on GnRH induced gonadotropin secretion in healthy women**

The kisspeptin neuronal population has marked sexual dimorphism, which in rodents, is particularly striking in the anteroventral paraventricular nucleus, with females having more Kiss1 neurons than males (41, 42). Sexually dimorphic expression is thought to be linked to sexually dimorphic traits such as the midcycle surge of gonadotropins, which is unique to the female. However, responsiveness to exogenous to kisspeptin administration also appears to be sexually dimorphic. Although kisspeptin is a powerful stimulus for LH release in men, its effects are much more variable in women and appear to depend on the phase of the menstrual cycle in which kisspeptin is administered. In the periovulatory and luteal phase females, administration of plasma kisspeptin in human females potently stimulates LH release, but in the follicular phase of the cycle, the response...
is much more muted, whether kisspeptin 68–121 or kisspeptin 112–121 is used (34, 43, 44) (Table 2). Although the reasons for these differences are not clear, one hypothesis is that the ambient level of endogenous kisspeptin may be higher in the follicular phase than other phases of the cycle such that the impact of a single exogenous bolus may have little effect (44). However, kisspeptin responsiveness is not simply determined by the relative levels of estrogen alone because postmenopausal women show higher gonadotropin responses to kisspeptin than follicular phase women, whereas healthy women on combined oral contraceptive pills show diminished responsiveness to exogenous kisspeptin (45, 61).

Table 2.
Responses to intravenous kisspeptin boluses in women (follicular phase)

Kisspeptin administration to hypogonadotropic patients

Kisspeptin has been administered not just to healthy volunteers but also to women with hypothalamic amenorrhea, in which patients develop an acquired form of hypogonadotropism, often, but not exclusively, in the setting of low body weight. Although sc injection of kisspeptin 68–121 stimulated gonadotropin secretion in these patients, these acute effects were lost when the same dose was administered twice daily over a 2-wk period, suggesting that chronic, nonpulsatile kisspeptin exposure may result in desensitization. However, biweekly administration of kisspeptin results in a sustained gonadotrophin response to kisspeptin (33). Thus, these human studies reveal the delicate balance of dose and exposure in maintaining responsiveness to this neuropeptide.

Another human model used to study acquired hypogonadotropism is the hypogonadism associated with obesity and type 2 diabetes. Many pathophysiological alterations in metabolic variables associated with this common clinical scenario have also been shown to down-regulate kiss1 expression in animal models. These findings led to the hypothesis that decreased endogenous kisspeptin tone plays a central role in the pathophysiology of the hypogonadotropism (46). Preliminary results show comparable responses to kisspeptin-10 boluses and infusions in obese hypogonadal men with type 2 diabetes are comparable with healthy volunteers (45), supporting the notion that the GnRH-gonadotrope-Leydig cell axis in these hypogonadotropic men per se is functionally intact.

Kisspeptin as a tool to unearth fundamental properties of GnRH pulse generation

Pulsatility is an intrinsic property of GnRH neurons because a clonal GnRH neuronal cell line can spontaneously produce pulses of GnRH (47). However, the possibility that a pulse generator may reside outside the GnRH neuron has been raised by lesioning experiments in nonhuman primates (48, 49) and rodents (50) that have localized such functionality to the mediobasal hypothalamus. Progesterone, endogenous opioids, and their pharmacological agonists and antagonists are all known modulators of GnRH pulse frequency (51–58). However, the nature of the rhythmogenicity of any endocrine clock, including the GnRH pulse generator, has heretofore, eluded investigators.

Kisspeptin administration has provided some new insights on the pulsatility of GnRH neurons. When kisspeptin is administered as a single bolus to men, it does not appear to change GnRH pulse frequency in the hours immediately after its administration. Stated simply, single boluses of kisspeptin do not speed up subsequent GnRH pulses. However, a single bolus of kisspeptin does reset the GnRH pulse generator (35). In other words, the kisspeptin-induced pulse, rather than being an extraepisodic event wedged in between endogenous LH pulses, is somehow detected by the GnRH neuronal network and used to calculate the timing of the subsequent endogenous pulse.

In contrast to single bolus kisspeptin administration, continuous kisspeptin administration via iv infusion has
more abiding effects on GnRH pulse frequency (30). It speeds it up. This is a surprising finding because in juvenile and adult male monkeys, continuous kisspeptin desensitizes the kisspeptin receptor and, by extension, results in marked drops in LH (59, 60). However, in the human male, continuous kisspeptin (at lower doses than what was used in the monkey) has not resulted in desensitization but rather in increases in both LH pulse frequency and size. Whether increases in the dose (body weight adjusted doses of kisspeptin-10 used in the human were about an order of magnitude lower) or duration of continuous kisspeptin (up to 22.5 h of kisspeptin-10 administered in men vs. 3 d in the monkey) will desensitize the kisspeptin receptor remains to be determined. Taken together with observations of decreased LH pulse frequency in animal models exposed to kisspeptin antagonists (24), increased LH pulse frequency observed in men exposed to continuous kisspeptin suggests a central role for KNDy neuronal network in the modulation of LH pulse frequency. However, whether physiological kisspeptin secretion in the human is pulsatile or not remains to be elucidated.

The approach of administering continuous kisspeptin has now been extended not only to normal volunteers but also to patients with mutations in genes that are important in the hypothalamic control of reproduction. As discussed earlier, mutations in the NKB pathway result in the phenotype of isolated GNRH deficiency and hypogonadotropic hypogonadism, and kisspeptin has now been administered to patients with NKB signaling defects. Continuous kisspeptin was shown to elicit stimulation of LH secretion (mean levels and pulsatile secretion) in four patients with mutations in the neurokinin signaling pathway (36). This suggests that kisspeptin signaling occurs downstream of neurokinin B in the functional hierarchy of neuroendocrine regulation of human reproduction and that pulsatile gonadotropin secretion can be modified by continuous kisspeptin administration in the setting of particular genetic defects.

Conclusions

Within a decade of the discovery of a reproductive role for kisspeptin, a large body of evidence has accrued from animal models and early translational clinical studies, providing novel insights into the functional regulation of GnRH neurons in physiological and pathological settings. Kisspeptin administration to human subjects with isolated GnRH deficiency will be a valuable physiological tool for unraveling the mystery of how pulses of GnRH secretion are generated, how these functional properties are modified in disease states, and how GnRH secretion may be modified to treat reproductive disorders.

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Footnotes

Abbreviations:

GPR54  G protein coupled receptor 54
KISS1R  kisspeptin-1 receptor
KNDy  kisspeptin-neurokinin B-dynorphin
NKB  neurokinin B.


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gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. Endocrinology 149:1979–1986 [PMC free article] [PubMed]


