Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling

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Abstract

The *Kiss1* gene codes for a family of peptides that act as endogenous ligands for the G protein-coupled receptor GPR54. Spontaneous mutations or targeted deletions of GPR54 in man and mice produce hypogonadotropic hypogonadism and infertility. Centrally administered kisspeptins stimulate gonadotropin secretion by acting directly on GnRH neurons. Sex steroids regulate the expression of KiSS-1 mRNA in the brain through direct action on KiSS-1 neurons. In the arcuate nucleus (Arc), sex steroids inhibit the expression of KiSS-1, suggesting that these neurons serve as a conduit for the negative feedback regulation of gonadotropin secretion. In the anteroventral periventricular nucleus (AVPV), sex steroids induce the expression of KiSS-1, implying that KiSS-1 neurons in this region may have a role in the preovulatory LH surge (in the female) or sexual behavior (in the male).

Reproduction (2006) **131** 623–630

Discovery

GPR54 is a G protein-coupled receptor, which was originally identified as an 'orphan' receptor in the rat (Lee *et al.* 1999). Although GPR54 shares a modest sequence homology with the known galanin receptors, galanin apparently does not bind specifically to this receptor (Lee *et al.* 1999). In 2001, three teams of investigators discovered in quick succession that the natural ligand for GPR54 is a 54-amino-acid product of a gene called *Kiss1* (Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001). The *Kiss1* gene was originally isolated as a tumor metastasis gene, and the peptide product was named metastatin, reflecting its ability to suppress metastasis of melanomas (Lee *et al.* 1996). (In this review, the term 'kisspeptin' will refer to metastatin and other biologically active fragments of metastatin.) In 2003, two independent groups discovered almost simultaneously that disabling mutations of GPR54 are associated with a failure to progress through puberty and hypogonadotropic hypogonadism in man (de Roux *et al.* 2003, Seminara *et al.* 2003). This observation was corroborated by studies of mice bearing targeted deletions of GPR54, where it was noted that reproductive dysfunction is apparently the only remarkable phenotypic anomaly associated with the mutation (Funes *et al.* 2003, Seminara *et al.* 2003). Thus, kisspeptin-GPR54 signaling is

essential to initiate gonadotropin secretion at puberty and support reproductive function in the adult.

How *Kiss1* got its name

Investigators at the Pennsylvania State College of Medicine in Hershey, Pennsylvania, discovered the *Kiss1* gene. To associate the discovery with their hometown and its most famous product – the Hershey chocolate Kiss – and to incorporate the letters 'SS' (referring to 'suppressor sequence'), the scientists named the gene KiSS-1. By the 'Rules for Nomenclature of Genes, Genetic Markers, Alleles, and Mutations in Mouse and Rat', the authorities at the Mouse Genome Informatics renamed the gene *Kiss1*. However, the revised nomenclature has not yet been widely adopted. In this review, we will refer to the gene as *Kiss1* and the messenger RNA as KiSS-1 mRNA, in keeping with current usage.

Kisspeptins stimulate gonadotropin-releasing hormone (GnRH)

Several groups have now shown that kisspeptin, administered either centrally or peripherally, stimulates gonadotropin secretion. Gottsch *et al.* (2004) reported that extraordinarily low doses of kisspeptin (~1 fmol), injected

into the lateral ventricle of the mouse, can elicit a rapid and robust secretory burst of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Similar observations (but with higher doses of kisspeptin) were reported in the rat (Matsui *et al.* 2004, Navarro *et al.* 2004a, 2005), sheep (Messenger *et al.* 2005), monkey (Shahab *et al.* 2005, Plant *et al.* 2006) and, most recently, the human male (Dhillon *et al.* 2005). In addition to corroborating the observation that kisspeptin stimulates gonadotropin secretion, these latter studies also demonstrated that the effect of kisspeptin on circulating levels of LH is unusually prolonged – far beyond that which would have been produced by centrally administered secretagogues, such as *N*-methyl-D-aspartate (NMDA) (Saitoh *et al.* 1991, Navarro *et al.* 2004a, 2005). Moreover, as demonstrated in the mouse, rat, and monkey, the GnRH antagonist acyline can block the kisspeptin-induced release of LH and FSH (Gottsch *et al.* 2004, Irwig *et al.* 2004, Shahab *et al.* 2005). This suggests that kisspeptin-stimulated gonadotropin release is dependent on the release of GnRH and does not reflect a direct action of kisspeptin on the pituitary. The apparent lack of kisspeptin's effect on the pituitary was confirmed in the rat by Matsui *et al.* (2004), who reported that another GnRH antagonist, cetorelix, also blocks the kisspeptin-induced release of LH and FSH. However, *in vitro*, kisspeptin has been shown to stimulate LH release and augment GnRH-stimulated FSH release from pituitary explants derived from peripubertal male rats – albeit modestly (Navarro *et al.* 2005a, 2005b); nevertheless, kisspeptin has little or no effect on the release of either LH or FSH from primary cultures of pituitary cells derived from rats (Matsui *et al.* 2004). Moreover, another study reports that kisspeptin has no effect on LH and FSH release from rat pituitary explants, yet it stimulates GnRH

secretion from hypothalamic explants (Thompson *et al.* 2004). The concept that kisspeptin can activate GnRH neurons in the forebrain is buttressed by reports that kisspeptin induces expression of the immediate early gene product Fos in GnRH neurons in the rat (Irwig *et al.* 2004, Matsui *et al.* 2004). Furthermore, infusion of kisspeptin into the third ventricle of sheep increases levels of GnRH found in the cerebrospinal fluid (Messenger *et al.* 2005). Despite compelling evidence that kisspeptins act directly on the hypothalamus to induce gonadotropin release, GPR54 is expressed in the pituitary (Kotani *et al.* 2001, Muir *et al.* 2001). Thus, further experiments are necessary to determine whether kisspeptin-GPR54 signaling in the pituitary plays a role in the regulation of either gonadotropin or other pituitary hormone secretion.

Kisspeptin-expressing neurons are localized in discrete regions of the forebrain. Early reports indicated that transcripts for KiSS-1 mRNA are detectable by RT-PCR in the human brain (Muir *et al.* 2001). Gottsch *et al.* (2004) mapped the location of cells expressing KiSS-1 mRNA in the mouse by *in situ* hybridization. They found that KiSS-1 mRNA is expressed in cells that reside in the anteroventral periventricular nucleus (AVPV), the periventricular nucleus (PeN), the anterodorsal preoptic nucleus (ADP) and the arcuate nucleus (Arc) (Gottsch *et al.* 2004, Smith *et al.* 2005a,b) (Fig. 1). Studies in the rat by immunocytochemistry have yielded mixed results. An initial report suggested that kisspeptin-containing cell bodies may be located in the dorsomedial hypothalamic nucleus, nucleus of the solitary tract, and the caudal ventrolateral medulla, with only scattered positive cells in the PeN and Arc (Brailoiu *et al.* 2005); however, a second published report testifies to observing kisspeptin-containing cell bodies only in the Arc (Kinoshita *et al.* 2005). Additional studies

Figure 1 Localization of KiSS-1 mRNA in the forebrain of the mouse. Each panel depicts a hypothalamic section. Panel A is most rostral and shows KiSS-1 mRNA localization (red dots) in the anteroventral periventricular nucleus (AVPV), periventricular nucleus (PeN) and anterodorsal preoptic area (ADP). Panel B is more caudal and shows KiSS-1 mRNA localization in the arcuate nucleus (Arc). DMC: dorsomedial hypothalamus (compact); DMD: dorsomedial hypothalamus (diffuse); ME: median eminence; VMHDM: ventromedial hypothalamus (dorsomedial); VMHVL: ventromedial hypothalamus (ventrolateral).

with other, better antisera to kisspeptin will be required to clarify this matter.

Kisspeptin appears to act directly on GnRH neurons to stimulate the secretion of GnRH. Areas where kisspeptin neurons reside, such as the Arc and AVPV, are known to send projections to the medial preoptic area, where there is an abundance of GnRH cell bodies (Canteras *et al.* 1994, Simonian *et al.* 1999). Kisspeptin-containing fibers project to these same areas (Brailoiu *et al.* 2005), and indeed, kisspeptin fibers appear in close approximation to GnRH neurons (Kinoshita *et al.* 2005). If kisspeptin neurons communicate *directly* with GnRH neurons, one would expect GnRH neurons to express GPR54. Parhar *et al.* (2004) isolated GnRH neurons from the cichlid fish *Oreochromis niloticus* by laser capture microscopy and identified GPR54 mRNA transcripts in these cells by real-time quantitative RT–PCR. They found that approximately 50% of GnRH neurons in these animals coexpress GPR54. In the rat, Irwig *et al.* (2004) used double-label *in situ* hybridization to show that the majority (>75%) of GnRH neurons in this species also coexpress GPR54 mRNA; subsequently, it was shown that in the mouse approximately 50% of GnRH neurons express GPR54 (Messenger *et al.* 2005). Our own studies in the mouse with high-resolution *in situ* hybridization suggest that the percentage of GnRH neurons coexpressing GPR54 in this species may be even greater (Han *et al.* 2005). The relatively high degree of GPR54/GnRH coexpression is similar to the fraction of GnRH neurons that express Fos after central administration of kisspeptin (Irwig *et al.* 2004, Matsui *et al.* 2004). Thus, kisspeptin appears to act directly on GnRH neurons to stimulate GnRH secretion (Fig. 2). In addition, GPR54 appears to be the only functional receptor for kisspeptins – at least in the context of gonadotropin secretion – since kisspeptin has no effect on LH or FSH secretion in mice that lack a functional GPR54 (Messenger *et al.* 2005). Although these observations clearly establish the ability of kisspeptin-GPR54-GnRH signaling to activate LH and FSH secretion under experimental conditions, they provide little insight into kisspeptin's functional significance.

Steroids regulate kisspeptins

Gonadal activity – including hormone production and gametogenesis – is regulated by the brain and pituitary, which are both targets for the feedback control of gonadotropin secretion by sex steroids. Although GnRH neurons appear to express the estrogen receptor β (ER β) (Hrabovszky *et al.* 2000, 2001), it is widely believed that other steroid-responsive neurons in the forebrain mediate the predominant actions of estrogen and androgen in the regulation of GnRH and gonadotropin secretion (Herbison 1998). ER α and ER β , as well as the androgen receptor (AR), are expressed in the periventricular regions of the forebrain where kisspeptin

Figure 2 Proposed interactions between kisspeptin-secreting neurons and GnRH neurons. In this model, KiSS-1 mRNA-expressing neurons, from the arcuate nucleus (Arc) and the anteroventral periventricular nucleus (AVPV), make synaptic contact with GnRH neurons within the preoptic area (POA). Upon activation of the kisspeptin receptor GPR54, GnRH neurons are stimulated to release GnRH into the portal circulation, which in turn stimulates the release of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the pituitary.

neurons reside (Simerly *et al.* 1990, Hagihara *et al.* 1992, Shughrue *et al.* 1997). The phenotypic identity of cells that receive input from gonadal steroids and relay this information to GnRH neurons remains unknown; however, kisspeptin neurons seem poised to play this role. Measurements by RT–PCR reveal that total hypothalamic content of KiSS-1 mRNA increases significantly after gonadectomy and decreases with sex steroid replacement (Navarro *et al.* 2004a). However, a detailed, regional analysis of KiSS-1 mRNA in the forebrain of mice by *in situ* hybridization has produced a more complex picture. In the Arc, manipulations of the sex steroid milieu by castration and sex steroid replacement produce the same outcome as reflected by RT–PCR measurements – an increase in KiSS-1 mRNA after castration and decline with steroid treatment. However, in the AVPV and PeN, the opposite occurs in both sexes – a *decline* in the expression of KiSS-1 mRNA with castration and an *increase* in expression with sex steroid replacement (Smith *et al.* 2005a, 2005b) (Fig. 3). Thus, the activity of kisspeptin neurons in the Arc is stimulated by gonadectomy and inhibited by sex steroids. If kisspeptin neurons in the Arc provide tonic facilitatory input to GnRH neurons, it seems plausible that kisspeptin neurons could mediate the negative feedback effects of steroids on GnRH secretion, activating GnRH neurons when plasma levels of sex steroids decline and inhibiting GnRH neurons when sex steroids rise.

AVPV

Arc

Figure 3 KiSS-1 mRNA is differentially regulated by estrogen in the forebrain of the mouse. The upper panel depicts the expression of KiSS-1 mRNA (red dots) in the anteroventral periventricular nucleus (AVPV) of the female diestrus (intact), ovariectomized (OVX), and OVX-estradiol replaced (+E) mouse. The diagram illustrates the apparently positive (+ve) regulation of KiSS-1 mRNA by E in the AVPV. The lower panel depicts the expression of KiSS-1 mRNA in the arcuate nucleus (Arc) of the female diestrus (intact), OVX and OVX + E mouse. The diagram illustrates the apparently negative (–ve) regulation of KiSS-1 mRNA by E in the Arc.

The KiSS-1 neurons that reside in the AVPV clearly behave differently from those in the Arc; hence, KiSS-1 neurons in this region would appear to serve a different physiologic function. The AVPV is one of the few sexually dimorphic areas of the forebrain that is larger in the female than the male (Simerly 1998), and in this region there are many more KiSS-1 neurons in the female than the male (Smith *et al.* 2005a,b). The AVPV has been implicated in the generation of the preovulatory GnRH/LH surge in the female (Gu & Simerly 1997). The phenotypic identity of neurons in the AVPV that help to orchestrate the surge is unknown, but kisspeptin neurons could conceivably serve this function. Sex steroids induce the expression of KiSS-1 in the AVPV, and it seems plausible that this activational event drives the GnRH/LH surge. Indeed, central administration of an 'antikisspeptin' antibody blocks the LH surge at proestrus in the rat (Kinoshita

et al. 2005), indicating that kisspeptin is essential for generating the preovulatory LH surge. This interpretation would not rule out a contribution by KiSS-1 neurons in the Arc, which apparently show an increase in peptide and Fos expression (detected by immunocytochemistry) at the time of proestrus (Kinoshita *et al.* 2005). Further studies are required to clarify the potential role of kisspeptin neurons in the AVPV and Arc in generating the preovulatory GnRH/LH surge.

The involvement of gonadal steroids in the regulation of kisspeptin neuronal activity suggests that either kisspeptin neurons express steroid receptors or they receive input about circulating steroid levels from other steroid-sensitive neurons. Smith *et al.* (2005b) showed that cells expressing KiSS-1 mRNA also express sex steroid receptors. In male mice, more than 60% of KiSS-1 neurons in the Arc express AR and about 90% express

the ER α . In the female mouse, nearly all KiSS-1 neurons express ER α , and approximately 30% express ER β (Smith *et al.* 2005a). Thus, KiSS-1 neurons are direct targets for the action of sex steroids in both the male and female mouse.

Although it is reasonable to assume that the effects of estrogens on kisspeptin neurons are mediated by ER, testosterone can either act directly through AR or indirectly through ER after its aromatization to estradiol. To determine which steroid receptors are actively involved in the regulation of kisspeptins in the male, Smith *et al.* (2005b) examined the effects of estradiol and dihydrotestosterone (DHT), a nonaromatizable androgen, on the expression of KiSS-1 mRNA in castrated male mice. Estradiol mirrored the effect of testosterone in both the Arc and AVPV, whereas DHT had an effect only in the Arc. This would suggest that both AR and ER play a role in the regulation of KiSS-1 expression in the Arc, but that the ER mediates the effects of testosterone on KiSS-1 regulation in the AVPV. For further clarification of the role of the sex

steroid receptors in the regulation of KiSS-1 mRNA, the effect of steroid hormone treatments was assessed in mice bearing genetically targeted deletions (or crippling mutations) of the various receptors. In male mice, mutations in neither ER α nor AR altered the response of KiSS-1 to testosterone in either the Arc or AVPV (Smith *et al.* 2005b). Thus, in the male with a congenital absence of either ER α or AR, the remaining receptor can fully compensate for the lack of the other. This does not appear to be the case in the female. In the female mouse, targeted deletion of the ER α completely blocks the ability of estradiol to regulate the expression of KiSS-1 in both the AVPV and Arc (Fig. 4), whereas a genetically targeted deletion of ER β had no effect on the ability of estradiol to regulate KiSS-1 expression in either the Arc or AVPV (Smith *et al.* 2005a). Thus, in the female, it appears that ER α plays a critical role in the regulation of KiSS-1 in both the AVPV and Arc, and ER β plays no clearly discernible role in this process.

Figure 4 The effect of estradiol on KiSS-1 mRNA expression depends on estrogen receptor alpha (ER α). In wild-type (WT) control mice after ovariectomy, the expression of KiSS-1 mRNA (red dots) is reduced in the anteroventral periventricular nucleus (AVPV) and elevated in the arcuate nucleus (Arc). This pattern is reversed with estradiol (E) replacement; that is, KiSS-1 mRNA is elevated in the AVPV and reduced in the Arc. In ER α knockout mice, E treatment has no effect on the expression of KiSS-1 mRNA in either the AVPV or Arc.

Does kisspeptin-GPR54-GnRH signaling trigger puberty?

Animals with disabling mutations and targeted deletions of GPR54 fail to progress through puberty, as a result of hypogonadotropic hypogonadism (Funes *et al.* 2003, Seminara *et al.* 2003). This would argue that activation of GPR54 is at least 'permissive' for the onset and maintenance of GnRH and LH secretion; however, the precise role that kisspeptin-GPR54-GnRH signaling plays in gating the onset of puberty is unclear. Activation of GnRH neurons is the key event that initiates the onset of puberty (Ojeda & Urbanski 1994, Plant 1994), but the nature of this 'trigger' remains to be identified. Kisspeptin-GPR54 signaling is a plausible candidate. Matsui *et al.* (2004) demonstrated that peripheral administration of kisspeptin to prepubertal, 25-day-old female rats stimulates LH secretion and induces ovulation in the rat. Shortly thereafter, Navarro *et al.* (2004a, 2004b) demonstrated a similar effect of kisspeptin on LH secretion in prepubertal male rats and showed that kisspeptins could advance the timing of vaginal opening in females. If kisspeptins trigger puberty onset, one would expect to see an increase in KiSS-1 mRNA and/or GPR54 mRNA expression during this time. Using semiquantitative RT-PCR on hypothalamic fragments, Navarro *et al.* (2004a) showed that this is indeed the case in both male and female rats. In the male mouse, KiSS-1 mRNA expression, measured by *in situ* hybridization, is stable in the Arc across pubertal development but increases significantly in the AVPV (Han *et al.* 2005), a region directly implicated in the activation of

GnRH neurons (Herbison 1998). Thus, kisspeptin-GPR54 signaling appears to be amplified at the time of puberty and could represent the proximate event that activates GnRH neurons and awakens the neuroendocrine reproductive axis at puberty.

The physiologic mechanisms that govern the onset of puberty differ between the rodent and primate (Ojeda & Urbanski 1994, Plant 1994), yet recent evidence suggests that kisspeptin may also play a role in triggering the onset of puberty in the primate. First, central injections of kisspeptins stimulate LH in prepubertal, agonadal male monkeys, demonstrating that kisspeptin can override the central inhibition of GnRH secretion characteristic of the prepubertal primate (Shahab *et al.* 2005). Second, hypothalamic content of KiSS-1 mRNA increases across puberty in both the agonadal male and intact female monkey, suggesting that increased production of kisspeptin could contribute to activating the neuroendocrine reproductive axis at puberty in this primate species (Shahab *et al.* 2005). Hypothalamic levels of GPR54 mRNA also increase as a function of pubertal maturation – but only in the intact female – indicating that this is a steroid-dependent phenomenon and unlikely to be a centrally mediated 'triggering' event for puberty (Shahab *et al.* 2005).

The electrophysiologic response of GnRH neurons to kisspeptins appears to change dramatically over the course of puberty. With gramicidin-perforated patch recordings from brain slice preparations, approximately 30% of GnRH neurons responded to kisspeptin administration in prepubertal male GnRH-GFP transgenic mice,

Figure 5 Possible role of kisspeptin in the onset of puberty. Recent observations suggest that GnRH neurons become increasingly responsive to kisspeptin as a function of pubertal maturation in the mouse. In this example, gramicidin-perforated patch recordings were used to assess the electrophysiologic response of GnRH neurons to central kisspeptin administration (red bar).

whereas 90% of GnRH neurons from adult mice responded to the same dose of kisspeptins (Han *et al.* 2005) (Fig. 5). In addition, the excitatory effect of kisspeptin appears to directly activate GnRH neurons because the response remains in the presence of tetrodotoxin, and this is in direct agreement with nearly all GnRH neurons expressing GPR54 (Han *et al.* 2005). In parallel to this data, central injections of lower doses of kisspeptins (10–100 fmol) stimulate LH in adult, but not prepubertal, male mice (Han *et al.* 2005). Thus, it appears in the mouse that GnRH neurons become developmentally activated by kisspeptins over the course of puberty (Fig. 5). Just how this phenomenon occurs remains uncertain. One would expect that the expression of GPR54 in GnRH neurons would increase to facilitate the increased kisspeptin response, but this does not appear to be the case. Levels of GPR54 mRNA in GnRH neurons are almost identical between prepubertal and postpubertal male mice (Han *et al.* 2005). Thus, it appears likely that kisspeptin participates in the pubertal renaissance of GnRH secretion in the primate. However, the newly refined question remains – *what awakens the kisspeptin/GPR54/GnRH circuitry at the time of puberty?* Only time and further research will tell.

In summary, the past 2 years has brought kisspeptin and its receptor GPR54 to the forefront of the neuroendocrine control of the gonadotropin axis. A vast array of data in many species has clearly shown that kisspeptin is critically important to the release of GnRH, and this stimulation appears to occur directly at the GnRH neuron. Furthermore, recent data suggest that kisspeptin-secreting neurons (those expressing KiSS-1 mRNA) are the long-sought link between peripheral sex steroids and GnRH release, and it is these neurons in the Arc and AVPV that appear to be well placed for both negative and positive feedback control of GnRH. Finally, evidence is continually emerging that links kisspeptin-GPR54 signaling to the onset of puberty, and it is this question that is providing new and exciting challenges for the future.

Acknowledgements

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 19 October 2005

First decision 30 November 2005

Revised manuscript received 23 December 2005

Accepted 12 January 2006