Endocrine, *vol. 26, no. 3, 277–284, April 2005* 0969–711X/05/26:277–284/$30.00

KiSS-1 and GPR54 as New Players in Gonadotropin Regulation and Puberty

Ursula B. Kaiser[1] and Wendy Kuohung[1,2]

Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and [2]Department of Obstetrics and Gynecology, Boston University School of Medicine, Boston, MA 02118

The recent identification of loss-of-function mutations in the gene encoding GPR54, the receptor for the KiSS-1–derived peptides, kisspeptins, has highlighted a previously unrecognized pathway in the physiologic regulation of puberty and reproduction. Patients with loss-of-function mutations in GPR54 have idiopathic hypogonadotropic hypogonadism, and mice lacking GPR54 similarly fail to undergo puberty and have immature reproductive organs and low levels of sex steroids and gonadotropins. These observations have led to the hypothesis that kisspeptins activate hypothalamic GnRH release, thereby serving as a pivotal factor in the pubertal activation of the reproductive cascade. This hypothesis is supported by subsequent studies in rodent and primate models that have demonstrated localization of KiSS-1 mRNA in the hypothalamus, colocalization of GPR54 in GnRH neurons, GnRH-dependent activation of LH and FSH release by intracerebroventricular or peripheral administration of kisspeptin, and increased hypothalamic KiSS-1 and GPR54 mRNA levels at the onset of puberty. Taken together, these findings weave a compelling case for a role of the kisspeptin–GPR54 system in the activation of GnRH neurons at the time of pubertal awakening of the reproductive axis.

Key Words: Gonadotropin-releasing hormone; gonadotropins; puberty; kisspeptin; GPR54; KISS-1; G protein–coupled receptor.

Introduction

Gonadotropin-releasing hormone (GnRH) is a neurohormone central to the initiation of the reproductive hormone cascade. Formed in hypothalamic neurons, GnRH is released in a pulsatile manner into the hypophysial portal circulation to stimulate the coordinated biosynthesis and secretion of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) *(1)*. LH and FSH, in turn, stimulate gonadal function, including gametogenesis and gonadal hormone synthesis. Thus, GnRH plays a pivotal role in the coordination of reproductive events. The pulsatile release of GnRH occurs during late fetal and early neonatal development but is then suppressed until puberty, when reactivation of GnRH release is the key event triggering the onset of pubertal development and reproductive maturation *(2,3)*. The secretion of hypothalamic GnRH is therefore classically considered the initial step in the reproductive cascade. Nonetheless, although the pivotal role of GnRH in the reproductive hierarchy of mammals is undisputed, the episodic release of GnRH itself is in turn governed by the interplay of excitatory and inhibitory signals in the form of neurohormones and neurotransmitters acting at the level of the hypothalamus. A maturational switch resulting in the enhancement of excitatory signals together with lowering of inhibitory signals is believed to occur at puberty, reawakening of GnRH neuronal function. It is in this context that the previously unsuspected role of the KiSS-1/GPR54 system in the neuroendocrine control of reproductive function has recently emerged.

Received April 27, 2005; Accepted April 27, 2005.

Author to whom all correspondence and reprint requests should be addressed: Ursula B. Kaiser, MD, Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115. E-mail: ukaiser@partners.org

Structure and Localization of GPR54

GPR54 (also known as AXOR12 or hOT7T175) was first described as an orphan G protein–coupled receptor of the rhodopsin family, cloned initially from rat brain *(4)* and subsequently identified in the human *(5–8)*. This seven-transmembrane-domain receptor shares highest homology, about 45%, with the galanin subfamily of receptors (Fig. 1A). Its five exons and four introns contain an open reading frame of 1197 base pairs that encode a 398-amino-acid protein (1191 base pairs and 396 amino acids in rat). The amino acid sequence is highly conserved across species, with 95% homology between the rat and mouse and 82% between mouse and human (98% in the transmembrane domains). Despite the homology of GPR54 with the galanin receptors, several residues in the galanin receptor shown to be important for high-affinity galanin binding are not conserved in GPR54, consistent with its lack of galanin binding activity *(4)*. Localization by Northern blot, *in situ* hybridization, and quantitative RT-PCR show that GPR54 has a broad expression pattern, with high expression levels in the brain, particularly in the hypothalamus, midbrain, pons, medulla, hippocampus, and

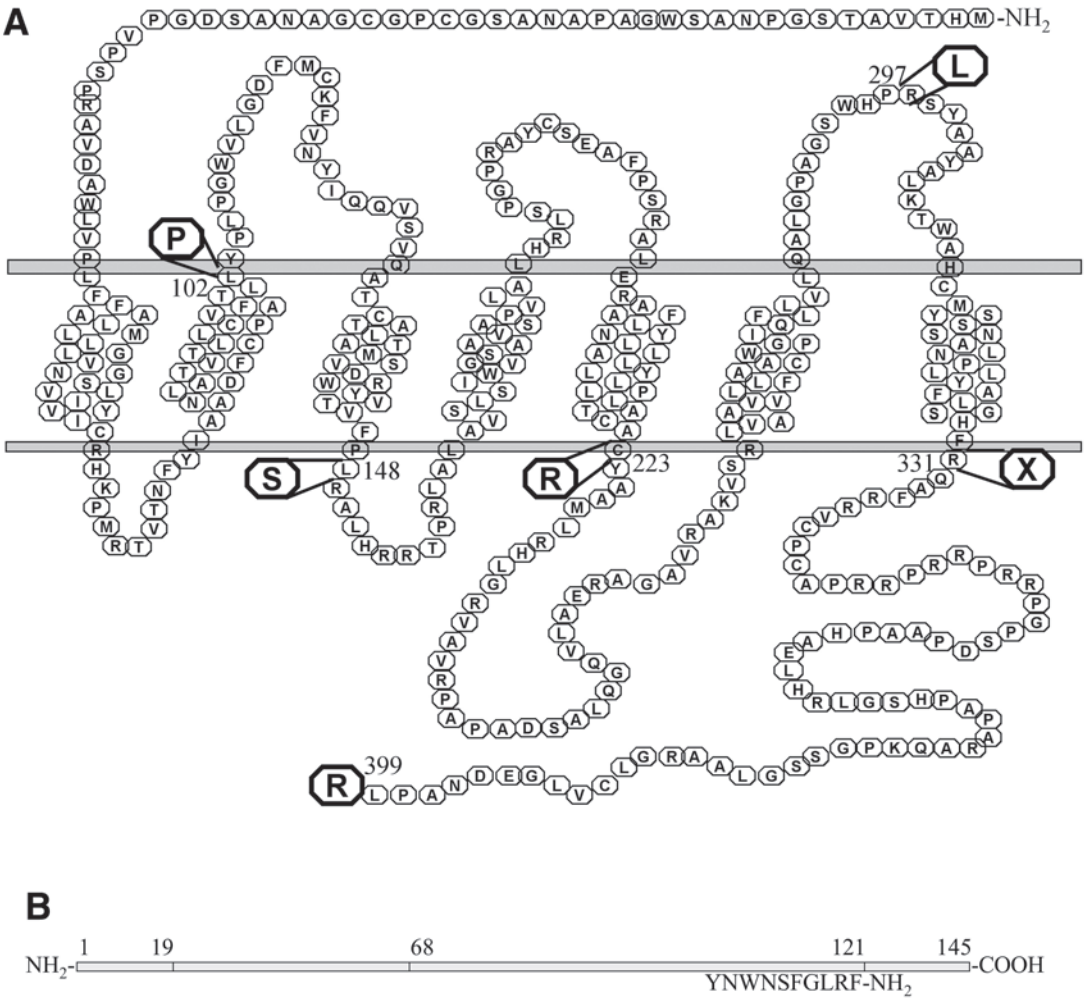

Fig. 1. (**A**) Model of the human GPR54 receptor. Mutations identified in patients with idiopathic hypogonadotropic hypogonadism are indicated. (**B**) Model of KiSS-1 protein product. Amino acids 1–19 are predicted to form a signal peptide. Proteolytic processing is predicted to produce kisspeptin-54, corresponding to amino acids 68–121. The C-terminal amidated decapeptide sequence, wherein biologic activity resides, is shown.

amygdala, and also in the pituitary, placenta, pancreas, and spinal cord, with lower levels in the heart, muscle, kidney, liver, intestine, thymus, lung, and testis *(4–8)*. Expression in the hypothalamus and pituitary is consistent with its newly recognized role in the neuroendocrine regulation of the reproductive axis.

Identification of Metastin/Kisspeptin, the Ligand for GPR54

Despite the homology with galanin receptors, the GPR54 receptor showed no activation in response to galanin or galanin-like peptide (GALP). A high-affinity ligand that binds to GPR54 was identified and described independently by four groups *(5–8)* as a peptide derived by proteolytic processing from the product of the *KiSS-1* gene *(9)*. *KiSS-1* encodes a 145-amino-acid (aa) peptide with features typical of secreted neuropeptides, including a signal sequence, several potential dibasic cleavage sites, and a cleavage/amidation site *(10)*. The predicted proteolytic processing results in a 54-amino-acid secreted peptide product (referred to as metastin or kisspeptin-54), corresponding to residues 68–121 of the *KiSS-1* gene product, and identical to the isolated peptide (Fig. 1B). Additional naturally occurring cleavage products of 13 and 14 amino acids also show biologic activity and correspond to the C-terminal amino acid sequences of kisspeptin-54 *(5,7,8)*. The shortest peptide tested that retains biologic activity is a decapeptide, corresponding to aa 112–121 of the human precursor peptide and referred to as kisspeptin-10 (Fig. 1B). Interestingly, all biologically active peptides display a C-terminal LRF-amide sequence. Other peptides that elicit a specific response via GPR54 include neuropeptides with a LRW/LRF-amide motif isolated from the sea anemone and the lobster *(7)*, suggesting that the GPR54 ligand derives from ancient origins. RF-amide peptides are widespread among invertebrates, are expressed in the central and peripheral nervous systems, function as neurotransmitters and neuromodulators, and have a diverse range of functions including control of feeding and reproduction *(6,11,12)*. More recently, a number of RF-

amides have been identified in mammalian species, including prolactin-releasing peptide and neuropeptides FF and AF *(13)*. These attributes are in keeping with the putative role of kisspeptin as a neuroendocrine stimulator of GnRH release.

Binding studies revealed a single high affinity binding site with K_d 1.0 ± 0.1 and 1.9 ± 0.4 n*M* for rat and human GPR54, respectively *(5,7)*. In binding and functional assays, kisspeptin-54, -14, and -13 all had similar or slightly reduced affinity and efficacy on the rat receptor compared to kisspeptin-10. Further N-terminal deletion of kisspeptin-10 resulted in a marked drop in functional potency. These observations suggest that the C-terminal 10 amino acids are the most important for receptor interaction. Stimulation of GPR54 by these peptides leads to activation of phospholipase C (PLC) with Ca^{2+} mobilization and phosphatidylinositol turnover, suggestive of coupling with proteins of the G_q class *(5,14)*. Stimulation of ERK and p38 phosphorylation and of arachidonic acid release is also observed. Pertussis toxin has no effect, suggesting that activation of $G_{i/o}$ proteins and subsequent $G_{\beta\gamma}$-mediated activation of PLCβ do not contribute to the observed Ca^{2+} response. In addition, there is no change in cAMP accumulation, indicating that this receptor also does not couple strongly to proteins of the G_s class.

KiSS-1 is highly expressed in the placenta, consistent with the biologic activity of placental extracts and purification of the peptides from placental tissues *(5,8,15,16)*. KiSS-1 mRNA is also expressed in testis, pancreas, liver, small intestine, and throughout the brain, particularly in the hypothalamus and basal ganglia, again consistent with its newly identified role as a modulator of GnRH release *(7,8)*.

Kisspeptin/Metastin in Cancer

Until recently, kisspeptin, also known as metastin, was defined based on its putative role in cancer metastasis. *KiSS-1* was originally identified as a human melanoma suppressor gene *(9,17)*. Subsequent studies showed that exogenous expression of *KiSS-1* in human melanoma and breast cancer cell lines suppressed metastasis *(17,18)*. Reduced invasiveness of HT-1080 fibrosarcoma transfected with *KiSS-1* was also reported *(19)*. The mechanism(s) of this antimetastatic activity is poorly understood. Ohtaki et al. showed that kisspeptin-54 inhibits cellular migration and induces an adhesive phenotype by increasing the phosphorylation of focal adhesion kinase and paxillin *(8)*. Further studies by this group found that metastin significantly suppresses cell motility in chemotaxis and wound-healing assays *(20)*. Metastin also inhibits spreading, monolayer growth, and colony formation, indicating that metastin is a potent inhibitor of cell motility but not cell adhesion, resulting in cell growth suppression and antimetastatic activity. The high levels of expression of kisspeptin and GPR54 in the placenta have led to the hypothesis that kisspeptin may regulate syncytiotrophoblast invasion in the uterus *(16,21)*.

Kisspeptin/GPR54 in the Control of Reproductive Function

In the context of investigation of the roles of kisspeptin and its cognate receptor GPR54 in cell migration and cancer, an unexpected role of this system in the regulation of reproductive maturation and function has emerged. In the human, loss-of-function mutations and deletions within the coding sequence of GPR54 have been identified in patients with idiopathic hypogonadotropic hypogonadism (IHH), a condition characterized by the absence of spontaneous pubertal development, low sex steroids, and inappropriately low gonadotropins, indicating that this receptor is absolutely required for puberty and implicating this pathway in the neuroendocrine control of reproduction *(22,23)*. In a large consanguineous Saudi family with six individuals with IHH *(24, 25)*, a homozygous single nucleotide change in exon 3 of GPR54 was found in all six affected individuals, resulting in substitution of a serine for the normal leucine in the second intracellular loop of the receptor (L148S) (Fig. 1A) *(22)*. This change did not occur in the homozygous state in any unaffected family members and was not identified in any controls. In addition, a normosmic IHH male was identified with compound heterozygous mutations in GPR54, with a single nucleotide change in exon 5 on one allele, replacing an arginine at residue 331 with a premature stop codon (991C>T [R331X]), and with another change in exon 5 on the other allele, replacing the stop codon at residue 399 with an arginine (1195T>A [X399R]), thereby resulting in the continuation of the open reading frame to the polyA signal, with no intervening stop codon (Fig. 1A). In a heterologous cell line transfected with wild-type or mutant GPR54 receptors, kisspeptin stimulation of inositol production, measured as an index of receptor function and activity, was reduced by all three mutants compared to wild-type GPR54. These studies confirmed that the amino acid changes identified in the subjects with IHH led to reduced biological activity of the GPR54 receptor.

In an independent finding, de Roux and colleagues similarly reported a large consanguineous family comprising five siblings with IHH *(23)*, with affected siblings of the family carrying a homozygous deletion of 155 nucleotides in GPR54, removing the splicing acceptor site of intron 4–exon 5 junction and part of exon 5. This deletion leads to receptor truncation within the third intracellular loop, resulting in the lack of transmembrane domains 6 and 7. A homozygous missense change (Leu102Pro) was identified by this group in an additional case of familial IHH (Fig. 1A), although the effects of this amino acid substitution on receptor function have not been studied in vitro. More recently, one additional subject with isolated IHH was reported to be a compound heterozygote harboring two sequence variations in GPR54: the first, a single nucleotide change in exon 4 resulting in the substitution of an arginine for the normal cysteine near the cytoplasmic end of the fifth transmem-

brane domain (C223R), and the second, a single nucleotide change in exon 5 leading to substitution of a leucine for the normal arginine in the third extracellular loop (R297L) (Fig. 1A) *(26)*. In a fluorometric calcium mobilization assay, the C223R mutant showed marked impairment of responsiveness to kisspeptin, although the R297L mutant had minimal effect, raising the possibility that this patient's GPR54 genotype may not fully explain his phenotype of complete IHH with bilateral cryptorchidism and micropenis evident at birth. Nonetheless, taken together, these studies have firmly established GPR54 as a newly recognized genetic determinant of sexual maturation and fertility. It is anticipated that studies of the mutations identified in such patients with reproductive disorders will lead to insights into the biochemical pathways controlling GPR54 and its ligand binding, signal transduction, and cellular actions.

Phenotype of *GPR54*$^{-/-}$ Mice

Mice homozygous for null mutations in GPR54 recapitulate the human phenotype of IHH *(27,28)*. *GPR54*$^{-/-}$ males had significantly reduced testicular size with hypoplastic Leydig cells and spermatogenic arrest, and they lacked secondary sex gland development of the preputial gland, the seminal vesicles, and the prostate. *GPR54*$^{-/-}$ females had small vaginal openings and sterility. Vaginal smears were similar to those observed in immature female mice and lacked evidence of an estrous cycle, uterine horns were threadlike, and ovaries were small, containing primary and secondary follicles but lacking corpora lutea or Graafian follicles *(22,28)*.

The homozygous mutant mice had hormonal profiles that faithfully recapitulated those of human IHH patients. *GPR54*$^{-/-}$ male mice had low testosterone levels, and *GPR 54*$^{-/-}$ female mice had estradiol levels comparable to those of normal mice at non-estrous stages of the reproductive cycle. Both males and females had low basal circulating gonadotropin levels. Most interestingly, hypothalamic extracts from these *GPR54*$^{-/-}$ mice showed normal GnRH protein content, similar to their wild-type counterparts, and *GPR54*$^{-/-}$ mice were able to secrete LH and FSH in response to GnRH injection. Thus, the GPR54-deficient mouse model provides key additional information regarding the locus of the defect. The normal hypothalamic content of GnRH in the *GPR54*$^{-/-}$ mice indicates that the pathways of GnRH neuronal migration and GnRH synthesis remain intact and suggests a defect in the processing or secretion of GnRH. The similarities between the mouse model and the IHH patients point to a role for GPR54 in the regulation of the hypothalamic–pituitary–gonadal axis and further establish GPR54 as a critical determinant of reproductive maturation across species.

Hypothalamic Expression of KiSS-1 and GPR54

The identification of mutations in GPR54 as a cause of IHH in humans and mice has led to studies to more precisely delineate the expression profiles of the KiSS-1 and GPR54 genes in the hypothalamus, to lend further support to the hypothesis that kisspeptin and GPR54 serve as regulators of GnRH release and are involved in the pubertal activation of the neuroendocrine system controlling GnRH neuronal activity. Earlier studies had already demonstrated the presence of GPR54 and KiSS-1 mRNA in the hypothalamus *(4–7)*. More detailed examination of the distribution of KiSS-1 mRNA in the mouse hypothalamus by *in situ* hybridization revealed KiSS-1 mRNA in the arcuate, periventricular, and anteroventral periventricular nuclei *(29)*, areas implicated in the neuroendocrine regulation of gonadotropin secretion *(30,31)*. Similarly, KiSS-1 mRNA has been shown to be expressed in cells in the region of the medial arcuate nucleus in primate models *(32)*. These patterns of distribution of KiSS-1 are consistent with a role of kisspeptin in the hypothalamic regulation of GnRH secretion, and supports kisspeptin as the physiologically relevant ligand for GPR54 in the neuroendocrine control of reproduction.

Given the presence of GPR54 in the hypothalamus, a major question has been whether GPR54 is expressed by GnRH neurons and whether kisspeptin acts directly on GnRH neurons. A report using laser capture microdissection of single digoxigenin-labeled GnRH neurons coupled with real-time quantitative RT-PCR demonstrated expression of GPR54 mRNA in GnRH neurons in tilapia, suggesting that the effects of kisspeptin may occur at least in part directly on GnRH neurons *(33)*. This observation, combined with the previously described effects of kisspeptin on cell migration, has led to speculation that activation of GPR54 by kisspeptin may serve as a "stop" signal for GnRH neuronal migration, leading to suppression of cell growth and modulation of GnRH secretion *(33)*.

The expression of GPR54 in GnRH neurons was confirmed in the rat, in which GnRH/GPR54 double-label *in situ* hybridization demonstrated that 77% of all GnRH mRNA-expressing cells also express GPR54 mRNA *(34)*. Similarly, GPR54 was shown to colocalize with GnRH-immunoreactive neurons in the preoptic area of mice *(35)*. Moreover, GPR54 expression was detected in the medial and lateral sections of the arcuate nucleus as well as in the ventral aspect of the ventromedial hypothalamus in primates *(32)*. However, GPR54 is also expressed in areas of the brain that do not contain GnRH neurons, suggesting that kisspeptin/GPR54 is likely to be involved in the regulation of other neurophysiological controls in addition to GnRH *(32,34)*.

Stimulation of LH Secretion by Kisspeptin

Further support for a role of the kisspeptin–GPR54 ligand–receptor system in the regulation of GnRH and gonadotropins comes from recent studies in which kisspeptin was administered in vivo in rodent and primate models. Several investigators have now demonstrated that administration of kisspeptin-10 or kisspeptin-54 results in a dose-depen-

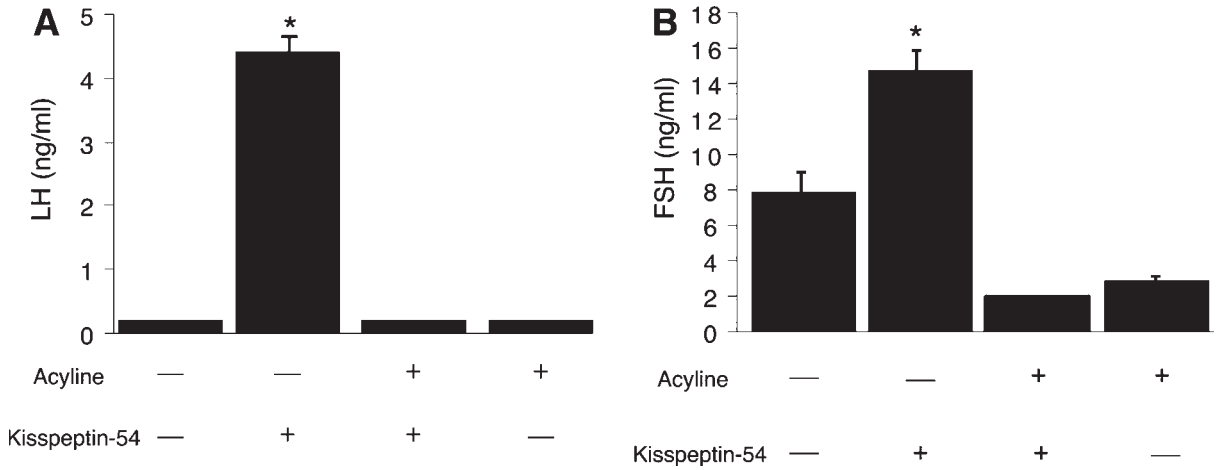

Fig. 2. The effects of intracerebroventricular kisspeptin-54 (50 pmol), with or without pretreatment with a GnRH antagonist, acyline (50 μg subcutaneously), on serum levels of (**A**) LH and (**B**) FSH in adult male mice *(29)*. (Reproduced with permission from Endocrinology, Copyright 2004, The Endocrine Society).

dent stimulation of LH release, in both mice and rats, in both males and females, and in prepubertal, pubertal, and adult rat models, as well as in agonadal juvenile male monkeys (Fig. 2) *(29,32,36–39)*. The effects of kisspeptin are dose-dependent, with an increase in serum levels of LH observed in adult male mice with doses of intracerebroventricular kisspeptin-54 as low as 1 fmol, demonstrating a remarkable potency of kisspeptin on LH release *(29)*. Increases in circulating LH levels were observed as early as 10 min and for as long as 4 h after ligand injection, with highest levels generally observed 15–60 min after intracerebroventricular administration in rats *(37,39)*. Similarly, in juvenile agonadal male monkeys, plasma LH levels increased by over 25-fold within 30 min of intracerebroventricular injection of kisspeptin-10 and remained elevated for 2–3 h, indicating that activation of hypothalamic GPR54 receptor signaling before puberty in primates induces precocious GnRH release *(32)*. Similar effects of kisspeptin on FSH levels were observed in rodents (Fig. 2), although the increase in FSH is somewhat more delayed *(29,34,36,39,40)*. Interestingly, kisspeptin-10 was found to be a less potent stimulator of FSH than LH, with ED_{50} values of 400 vs 4 pmol, respectively *(40)*. It can be speculated that kisspeptin may elicit dose-dependent patterns of pulsatile GnRH release that favor LH at lower concentrations. The stimulatory effects of kisspeptin on gonadotropins are observed after both central (intracerebroventricular) and systemic (intraperitoneal, subcutaneous, and intravenous) administration, which suggests an action on GnRH nerve terminals at the median eminence–arcuate nucleus complex, outside the blood–brain barrier. Following subcutaneous administration of kisspeptin-54, maximal increases in serum levels of LH and FSH occurred after 1–2 h *(36)*, whereas LH levels were increased 20 min after intraperitoneal kisspeptin-10 administration, but had returned to baseline by 60 min *(39)*. Whether these differences reflect the route of administration or differences in the rates of clearance or inactivation of kisspeptin-54 and kisspeptin-10 is not known. The administration of intraperitoneal kisspeptin to *GPR54*$^{-/-}$ male mice failed to induce any increase in plasma LH and FSH, confirming that the effects of kisspeptin on gonadotropins are mediated by GPR54 *(35)*.

The effects of intracerebroventricular kisspeptin were prevented by pretreatment with a GnRH antagonist in mouse, rat, and primate models, and the effects were mimicked by GnRH administration, with no additive effect when kisspeptin and GnRH administration were combined (Fig. 2) *(29,32,36,38,40)*. These observations suggest that the ability of kisspeptin to stimulate LH and FSH release is dependent upon GnRH and its receptor. From these data, we infer that kisspeptin acts on hypothalamic neurons that express GPR54 to regulate GnRH neuronal activity, thereby stimulating LH and FSH release. This hypothesis is further supported by the demonstration that administration of kisspeptin to hypothalamic explants stimulated the release of LHRH *(39)*. Whether this is a direct effect on GnRH neurons or whether it is mediated indirectly by intervening neurons remains to be determined, but the expression of GPR54 in GnRH neurons is strongly suggestive of a direct effect. Kisspeptin has been shown to stimulate transcriptional activity in GnRH neurons, as evidenced by the induction of c-Fos immunoreactivity *(34,36)*; 60–86% of GnRH neurons are positive for c-Fos immunoreactivity after kisspeptin administration, whereas <1% of GnRH neurons express c-Fos after injection of vehicle. Although kisspeptin is a potent stimulator of GnRH release, no effects on hypothalamic GnRH mRNA levels were detected following either acute or chronic intracerebroventricular administration of kisspeptin *(38)*. These results suggest that kisspeptin acts at the posttranscriptional level to stimulate the release of GnRH from GnRH neurons rather than affecting GnRH gene expression, consistent with the previously noted normal hypothalamic GnRH content noted in the *GPR54*$^{-/-}$ mice *(22)*. The recent report that infusion of kisspeptin into the third ventricle of sheep resulted in a rapid and sustained increase in GnRH levels

in the cerebrospinal fluid provides direct confirmation that kisspeptin administration induces GnRH release *(35)*.

To investigate the level of action of the KiSS-1/GPR54 system in the regulation of GnRH in hypothalamic pathways, the effects of kisspeptin in the presence of inhibitors of other pivotal neuroendocrine regulators of GnRH secretion, including excitatory amino acids, nitric oxide, and leptin have been studied *(38,40)*. Pharmacologic inhibitors of ionotropic glutamate receptors of both the NMDA and non-NMDA types and inhibitors of nitric oxide synthases had no effect on the LH or FSH secretory response to kisspeptin. Similarly, the LH and FSH responses to kisspeptin were preserved in three different models of leptin insufficiency: food deprivation, leptin immunoneutralization, and leptin resistance *(40,41)*. Thus, despite the key roles of the glutamate, nitric oxide, and leptin-mediated pathways in the control of GnRH neurons and in the regulation of puberty, kisspeptin/GPR54 is sufficient to activate GnRH-mediated gonadotropin secretion. Importantly, these observations point to a site of action of the KiSS-1/GPR54 system downstream of (or independent of) glutamate, nitric oxide, and leptin actions within the central circuitry governing GnRH release.

The marked increase in LH secretion following kisspeptin administration was reminiscent of the LH surge that triggers ovulation. Therefore, Matsui et al. investigated the effect of kisspeptin on PMSG-primed 25-d-old female rats, which have mature preovulatory ovarian follicles. In this model, kisspeptin administration was effective in triggering ovulation, with effects comparable to those of hCG, thereby linking kisspeptin administration to activation of the ovaries *(36)*. Similarly, in pubertal and adult male rats, testosterone levels were significantly increased following a single injection of either intracerebroventricular or intraperitoneal kisspeptin, demonstrating testicular activation *(38,39)*. Moreover, the intracerebroventricular administration of 1 nmol kisspeptin to prepubertal female rats every 12 h from age 26–31 d induced vaginal opening, a widely accepted marker of puberty, in 14 out of 19 treated females (74%) by age 31 d, whereas none of the vehicle-injected control females showed complete vaginal opening by this age (mean age of vaginal opening in controls 34.8 ± 0.35 d) *(41)*. The accelerated vaginal opening was associated with additional indices of pubertal maturation including increases in serum LH and estradiol levels and uterine weight, providing strong support for the hypothesis that central kisspeptin can trigger the onset of puberty.

Developmental and Hormonal Regulation of *KiSS-1* and *GPR54* Gene Expression

A recent study has demonstrated developmental and hormonal regulation of *KiSS-1* and *GPR54* gene expression in the rat hypothalamus *(37)*. Using both semiquantitative RT-PCR and real time RT-PCR, KiSS-1 and GPR54 mRNAs were detected in the rat hypothalamus throughout postnatal development, with the highest expression levels occurring at the time of puberty in both male and female rats. In addition, levels varied across the estrous cycle in females, with highest levels of both KiSS-1 and GPR54 mRNA during diestrus. These observations suggest an influence of gonadal steroids, which was confirmed by the demonstration of an increase in both KiSS-1 and GPR54 mRNA levels following gonadectomy in both male and female rats, an effect that could be prevented by the administration of testosterone or estradiol at the time of gonadectomy *(37)*. Castration of adult male rats increased KiSS-1 mRNA in the arcuate nucleus by both increasing the number of KiSS-1 neurons and increasing the cellular content of KiSS-1 mRNA *(34)*. In the agonadal male monkey model, hypothalamic content of KiSS-1 mRNA but not GPR54 mRNA was significantly higher in pubertal monkeys than in juvenile monkeys, and in female monkeys, both KiSS-1 and GPR54 mRNA levels in the medial basal hypothalamus were higher in midpubertal monkeys than in juvenile or early pubertal groups *(32)*. These findings suggest that both components of the kisspeptin–GPR54 ligand–receptor system in the hypothalamus may contribute to activation of the gonadotropic axis at puberty as well as its regulation in adulthood. KiSS-1/GPR54 may act as a trigger of puberty, or alternatively may have a permissive action on pubertal development.

Direct Effects of Kisspeptin on Pituitary Gonadotropin Secretion

The studies to date support a primary hypothalamic action of KiSS-1/GPR54 on stimulation of GnRH release. Nonetheless, the presence of high levels of GPR54 mRNA in the pituitary suggests a possible additional mechanism whereby kisspeptin and GPR54 may directly affect gonadotropin secretion, either independently or by modulating responses to GnRH *(4–7)*. Indeed, effects of kisspeptin have been observed on GnRH-stimulated, but not on basal, FSH release in cultured primary rat pituitary cells *(40)*. Similar effects were observed on LH secretion, in this case in the absence of GnRH *(38)*. On the other hand, others have observed no direct effect of kisspeptin on LH or FSH secretion in cultured primary rat pituitary cells and anterior pituitary fragments *(36,39)*. The different outcomes of these studies may reflect differences in the experimental paradigms, and the role of kisspeptin–GPR54 in the direct regulation of gonadotropins remains uncertain, requiring further investigation. While the major actions of kisspeptin appear to occur at the level of the hypothalamus to regulate GnRH release, potential modifier effects at the level of gonadotropin secretion in response to GnRH cannot be excluded.

Summary

The recent identification of loss-of-function mutations in *GPR54* has generated a new focus of attention on a previously unrecognized pathway in the physiologic regulation

of puberty and reproduction. This newly recognized function for GPR54 and its cognate ligand, kisspeptin, has led to additional studies that have demonstrated localization of KiSS-1 mRNA in the hypothalamus, colocalization of GPR54 in GnRH neurons, GnRH-dependent activation of LH and FSH release by kisspeptin, and increased hypothalamic KiSS-1 and GPR54 mRNA levels at the time of puberty. Taken together, these findings make a compelling case for a role of the kisspeptin–GPR54 system in the stimulation of GnRH neurons at the time of pubertal activation of the reproductive axis.

Nonetheless, many questions remain about this newly identified system. What are the mechanisms by which kisspeptin–GPR54 activate GnRH release? How are these signals integrated with other excitatory and inhibitory influences on GnRH neurons? What are the regulators of KiSS-1 and GPR54 and how is this pathway activated at the time of puberty? Is kisspeptin an endocrine hormone, a paracrine factor, or a neurotransmitter—or does it serve in all three capacities? What other functions might kisspeptin/GPR54 serve? With regard to this last question, it is worth remembering that kisspeptin has been found to stimulate oxytocin release *(5)* and to inhibit prolactin secretion *(37)*. No effects on food intake or on locomotive or sleeping behavior have been observed *(39)*. The previously recognized effects of kisspeptin on cell migration, adhesion, and chemotaxis have raised speculation that kisspeptin may serve as a signal to terminate GnRH neuronal migration in the hypothalamus and lead to suppression of cell growth and stimulation of GnRH secretion. The high levels of placental expression have led to similar hypotheses that kisspeptin may regulate trophoblast invasion in the uterus *(16,21)*. Potential roles in cancer biology and regulation of tumor metastasis remain an important consideration. Given the recent explosion of new information about this important ligand-receptor system, I anticipate that many of these questions will soon be answered.

Acknowledgment

This work was supported by NICHD/NIH through cooperative agreement U54 HD28138 as part of the Specialized Cooperative Centers Program in Reproduction Research. The authors thank Drs. Stephanie Seminara and William Crowley, Jr. (Massachusetts General Hospital) for their helpful discussions and comments.

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