

The Role of Kisspeptins and GPR54 in the Neuroendocrine Regulation of Reproduction

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metastin, *Kiss1*, GnRH, gonadotropin, estrogen

Abstract

Neurons that produce gonadotropin-releasing hormone (GnRH) reside in the basal forebrain and drive reproductive function in mammals. Understanding the circuitry that regulates GnRH neurons is fundamental to comprehending the neuroendocrine control of puberty and reproduction in the adult. This review focuses on a family of neuropeptides encoded by the *Kiss1* gene, the kisspeptins, and their cognate receptor, GPR54, which have been implicated in the regulation of GnRH secretion. Kisspeptins are potent secretagogues for GnRH, and the *Kiss1* gene is a target for regulation by gonadal steroids (e.g., estradiol and testosterone), metabolic factors (e.g., leptin), photoperiod, and season. *Kiss1* neurons in the arcuate nucleus may regulate the negative feedback effect of gonadal steroids on GnRH and gonadotropin secretion in both sexes. The expression of *Kiss1* in the anteroventral periventricular nucleus (AVPV) is sexually dimorphic, and *Kiss1* neurons in the AVPV may participate in the generation of the preovulatory GnRH/luteinizing hormone (LH) surge in the female rodent. *Kiss1* neurons have emerged as primary transducers of internal and environmental cues to regulate the neuroendocrine reproductive axis.

Kiss1: encodes kisspeptin-145, which is cleaved to become kisspeptin-54 (metastin)

DISCOVERY OF *KISS1*, KISSPEPTINS, AND THEIR RECEPTOR

Kisspeptins comprise a family of neuropeptides that are derived from the *Kiss1* gene. The *Kiss1* gene was so named in part because it was cloned in Hershey, Pennsylvania, a city known for its chocolate kisses (1, 2). The initial product of the *Kiss1* gene is a 145-amino-acid peptide. This peptide is cleaved into a 54-amino-acid peptide known as kisspeptin-54. Shorter peptides (kisspeptin-

10, -13, and -14) that share a common RF-amidated motif with kisspeptin-54 also exist. However, because no cleavage sites that would lead to the endogenous synthesis of these shorter derivatives have been detected, the shorter peptides may be degradation products of kisspeptin-54 (3–5) (**Figure 1**). Human kisspeptin-54 is also known as metastin, which reflects its ability to inhibit cancer metastasis (5). *Kiss1* expression was found initially in nonmetastatic cancer cells, which led to the discovery that kisspeptin could suppress the

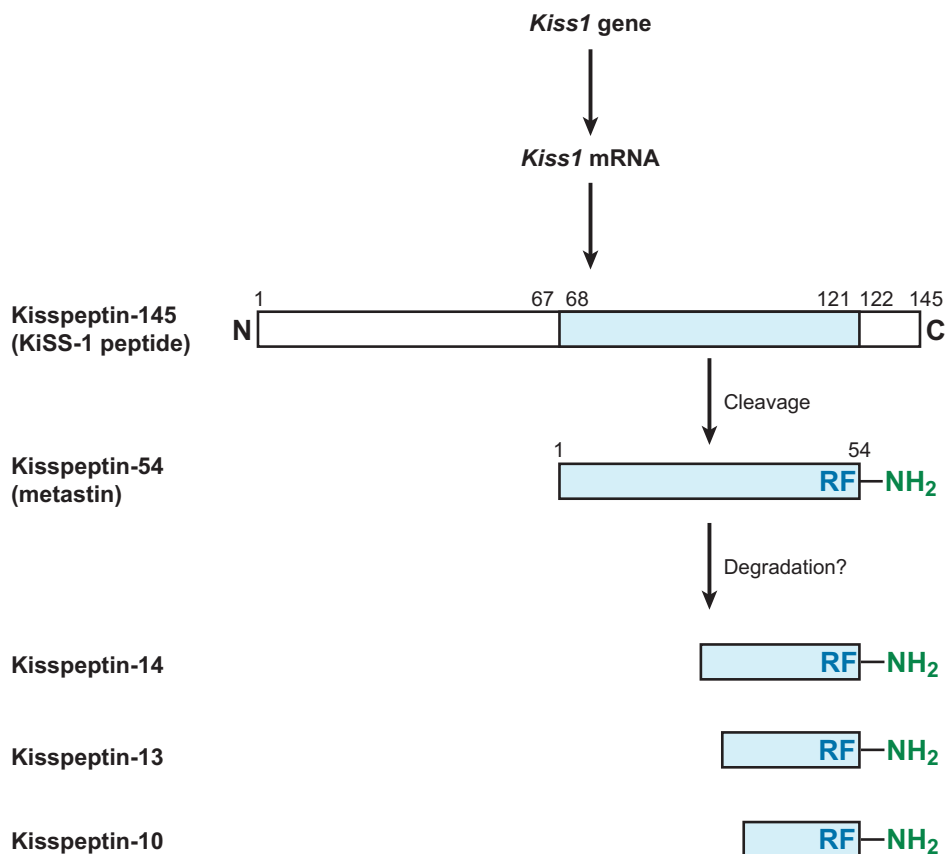


Figure 1

Products of the *Kiss1* gene. *Kiss1* mRNA is transcribed from the *Kiss1* gene and translated to form a 145-amino-acid propeptide called kisspeptin-145 or the KiSS-1 peptide. Shown are cleavage sites on the propeptide that lead to the production of the RF-amidated kisspeptin-54, also known as metastin. Shorter peptides (such as kisspeptin-10, -13, and -14) were identified by mass spectrometry. These peptides share a common C terminus and RF-amidated motif with kisspeptin-54. Because no putative cleavage sites have been identified on the propeptide that would lead to synthesis of the shorter peptides, such peptides may be degradation products of kisspeptin-54.

metastasis of malignant melanoma cell lines (1, 2) and breast carcinoma cell lines injected into mice (6). The role of *Kiss1* in cancer metastasis has been recently reviewed (7) and is not considered further in this review. *Kiss1* mRNA is also expressed in normal tissues such as the brain and other organs. Peripherally, *Kiss1* mRNA is most highly expressed in the placenta, with some expression in the testes, pancreas, liver, and intestines (5).

Researchers identified the kisspeptin receptor as the formerly orphan G protein-coupled receptor GPR54 (4, 5, 8–10). GPR54 has weak homology to galanin receptors (44–45%) but does not bind either galanin (9) or galanin-like peptide (5). *GPR54* mRNA is expressed in the brain, including the hypothalamus (9), and in a variety of other organs, such as the pituitary (4), placenta, and pancreas (5). The first evidence that kisspeptins activate GPR54 came from the discovery that placental extract could increase intracellular $[Ca^{2+}]$ in Chinese hamster ovary (CHO)-K1 cells that express GPR54 (4, 5). Investigators subsequently isolated kisspeptins from the human placenta, and all (kisspeptin-10, -13, -14, and -54) activate human and rat GPR54 in CHO-K1 cells (4).

GPR54 signals through a G_q -type of G protein. Kisspeptin stimulates phosphatidylinositol (PI) turnover, calcium mobilization, and arachidonic acid release in *GPR54*-expressing CHO-K1 cells (4). Kisspeptin treatment of COS-7 cells transfected with *GPR54* increases intracellular inositol 1,4,5-trisphosphate (IP_3) production (11), which suggests activation of the phospholipase C β (PLC β) pathway. In CHO-K1 cells, kisspeptin treatment leads to phosphorylation of extracellular signal-regulated protein kinase 1 (ERK1) and ERK2 mitogen-activated protein (MAP) kinases (4). Activation of MAP kinase may depend on G protein activation, although some G protein-coupled receptors stimulate MAP kinase in a G protein-independent manner (12). GPR54 activation may also lead to cytoskeletal reorganization. Kisspeptin treatment of COS-7 cells

that express *GPR54* changes cell morphology, possibly through an alteration in actin filament organization (11). Consistent with these findings, kisspeptin-10 stimulates stress fiber formation in *GPR54*-expressing CHO-K1 cells—perhaps through the Rho family of G proteins (4).

In 2003, two independent groups discovered that GPR54 plays an essential role in normal reproduction and pubertal development (13, 14). De Roux and coworkers (13) and Seminara and colleagues (14) found that humans with homozygous mutations in *GPR54* develop classic symptoms of idiopathic hypogonadotropic hypogonadism (IHH), which they discovered when patients sought treatment for infertility. Males with *GPR54* mutations have small testes, sparse pubic hair, retarded bone growth (13), and abnormally low circulating levels of gonadotropins and sex steroids (14). One affected woman had partial hypogonadism as well as low plasma levels of estrogen (E) and gonadotropins, partial breast development, and one episode of uterine bleeding at age 16 (13). Unlike patients with Kallaman's syndrome, patients with homozygous *GPR54* mutations are normosmic, which suggests that there are no major deficits in the embryonic migration of either primary olfactory neurons or gonadotropin-releasing hormone (GnRH) neurons (14, 15). Individuals with these mutations have normal anterior pituitary function (except for low gonadotropin production), and they respond to treatment with GnRH by showing increased circulating levels of gonadotropins (14). These findings suggest that GPR54 is required to initiate and maintain normal sexual maturation at puberty.

The phenotype of mice that bear a targeted null mutation in *GPR54* is similar to that of humans with mutations in *GPR54* (14, 16). *GPR54* knockout (KO) males have disrupted spermatogenesis, poorly developed secondary sexual characteristics, small testes, and low levels of testosterone (T). *GPR54* KO males also do not display mounting behavior. *GPR54* KO females have small vaginal openings and

GPR54: a G protein-coupled receptor that binds kisspeptins

E: estrogen

GnRH: gonadotropin-releasing hormone, also known as luteinizing hormone-releasing hormone (LHRH)

Testosterone (T): binds the androgen receptor; is converted to estrogen by aromatase

Estrous cycle: cycle of ovulation and reproductive behavior of most placental females, including the rat, mouse, and sheep

LH: luteinizing hormone

FSH: follicle-stimulating hormone

OVX: ovariectomized

low circulating levels of E and lack large developed follicles and corpora lutea. They do not become pregnant after mating and show little or no evidence of estrous cyclicity. Like humans with similar mutations, *GPR54* KO mice have lower plasma levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) than do their wild-type counterparts. Nevertheless, some gonadotropin secretion remains, demonstrating that pituitary gonadotropes are at least marginally functional in the *GPR54* KO mice. Furthermore, the hypothalamic content of GnRH and GnRH neuronal morphology are normal in *GPR54* KO mice (14, 17), which suggests normal GnRH neuronal migration and GnRH synthesis. Female *GPR54* KO mice respond to GnRH injections by showing an increase in plasma levels of LH and FSH, as do humans with similar mutations. *GPR54* KO mice show no obvious phenotypic deficits in either the number or type of cells in the primary sexual organs. *GPR54* KO males have both Leydig cells and seminiferous tubules, which suggests that the deleterious effects of *GPR54* disruption occur postnatally (16). Neither humans nor mice with mutations in *GPR54* have gross congenital abnormalities, except those that affect the reproductive axis. Finally, mice bearing targeted deletions of the *Kiss1* gene recapitulate the phenotype of the *GPR54* KO, which underscores the critical role of kisspeptin-GPR54 signaling in reproduction (18).

KISSPEPTINS EXCITE GnRH NEURONS

GnRH neurons receive inputs from a variety of neurotransmitters and neuromodulators. Thus, it is not surprising that central administration of numerous classical neurotransmitters and neuropeptides alters gonadotropin secretion through a GnRH-dependent pathway (19). Some of these inputs appear to play a direct role in shaping GnRH pulsatility (such as GABA, glutamate, and perhaps β -endorphin), whereas others provide permis-

sive or facilitatory signals to the GnRH network [such as norepinephrine and neuropeptide Y (NPY)] (19). These transmitters alter GnRH secretion by acting on receptors located on GnRH neuron cell bodies, dendrites, or terminals.

The seminal findings of de Roux and coworkers (13) and Seminara and coworkers (14) in 2003 raised the possibility that kisspeptin might stimulate GnRH secretion by acting through GPR54. Since then, several groups have demonstrated that central administration or peripheral administration of kisspeptin stimulates gonadotropin secretion through a GnRH-dependent mechanism (17, 20–24). In the male mouse, intracerebroventricular (ICV) administration of kisspeptin-54 and kisspeptin-10 evokes LH and FSH secretion at astonishingly low doses (21). Other studies that involved various delivery modes (e.g., ICV, subcutaneous, intravenous) showed similar effects of kisspeptin in the male and female rat (22, 23), ovariectomized (OVX) female sheep (17), juvenile male monkey (24–26), and human adult male (20).

Two lines of evidence suggest that kisspeptins increase gonadotropin secretion by stimulating GnRH secretion. First, pretreatment with the GnRH antagonists acyl-line (21, 22, 24) or cetrorelix (23) prevents kisspeptin-induced gonadotropin secretion. Second, ICV administration of kisspeptin increases GnRH secretion (as measured in cerebrospinal fluid) concurrent with LH secretion in female sheep (17). Kisspeptin appears to stimulate gonadotropin release by acting on GPR54 alone, because kisspeptin does not elicit gonadotropin release in the *GPR54* KO mouse (17).

Stimulation of LH secretion eventually ceases when animals are exposed to continuous kisspeptin infusion. In castrated juvenile male monkeys, whose pituitaries have been sensitized to GnRH, continuous intravenous infusion of kisspeptin-10 initially stimulates LH secretion, with peak levels appearing 1–2 h after the onset of the infusion; however, as exposure to kisspeptin continues, LH

levels decline to pretreatment levels by 12 h into the infusion (26). After four days of continuous kisspeptin infusion, GnRH is still capable of stimulating LH secretion, which suggests that desensitization occurs at the kisspeptin receptor, GPR54—not the GnRH receptor. In contrast, intermittent infusions of kisspeptin-10 stimulate pulsatile, GnRH-driven LH and FSH secretion for the duration of a 48-h experiment (25), suggesting that pulsatile kisspeptin release is capable of sustaining pulsatile GnRH and LH release. These results suggest that endogenous kisspeptin may induce GnRH secretion under normal physiological conditions.

Kisspeptin expression has been identified in hypothalamic nuclei important in the regulation of GnRH secretion. In situ hybridization (ISH) studies consistently localize *Kiss1* mRNA expression in the arcuate nucleus of the hypothalamus (ARC) of all mammalian species examined so far—including male and female mouse (21, 27), male and female rat (22, 28), male Syrian hamster (28, 29), male and female rhesus monkey (24), female macaque, female human (30), and female sheep (31). In the sheep, neurons in the ARC that contain kisspeptin also express dynorphin and neurokinin B (32), and indirect evidence suggests that this may also be true in primate species (33). In the female mouse and rat, *Kiss1* mRNA is expressed in the anteroventral periventricular nucleus (AVPV) (27, 28), with minimal expression in this nucleus in the male counterparts (34, 35).

Kisspeptin was also found by immunohistochemistry (IHC) in the ARC and AVPV of the mouse (36), with higher expression in the AVPV of the female as compared with the male. Kisspeptin-containing cell bodies also appear in the dorsomedial hypothalamus (DMH) of the rat, hamster, and mouse (36–38); however, to date no one has reported the presence of *Kiss1* mRNA in this nucleus as detected by ISH. This apparent discrepancy between the result obtained by IHC for kisspeptin and that by ISH for *Kiss1* mRNA may reflect differences in the sensitivity of

the assays. Alternatively, the kisspeptin antibodies used in the IHC studies may cross-react with peptides that share a similar C-terminal RF-amidated motif with kisspeptins but are not actual products of the *Kiss1* gene. In fact, Greives and coworkers (38) showed by IHC that kisspeptin immunoreactivity disappears in the DMH of the hamster when the antibody is preadsorbed with gonadotropin-inhibitory hormone (GnIH), which may explain some earlier reports showing the presence of kisspeptin in the DMH.

Kisspeptin-expressing cells are also found in the amygdala of the mouse (by ISH for *Kiss1* mRNA) (21), the nucleus of the solitary tract, and the caudal ventrolateral medulla of the rat (by IHC for kisspeptin) (37). Although the functions of kisspeptin in other regions of the brain are unclear, the identification of kisspeptin-expressing cell bodies in the rostral hypothalamus suggests that kisspeptin plays a role in the regulation of GnRH secretion (36). GnRH neurons express *GPR54* mRNA. The majority of GnRH neurons in the rat (>75%) express *GPR54* (22). Similar findings were reported in the cichlid fish (39), rhesus monkey (40), and mouse (17). The conserved expression of *GPR54* in GnRH neurons across vertebrate species suggests that the physiological role of this receptor is evolutionarily conserved and fundamental to reproduction.

Reverse transcription PCR (RT-PCR) studies also showed *GPR54* mRNA in the arcuate/median eminence (ME) of the rat (41). Such findings may reflect the expression of *GPR54* mRNA in GnRH axons or terminals or perhaps even other cell groups in the ARC. It is conceivable that *GPR54* mRNA is transported down the axon and translated in axon terminals of GnRH neurons, because the mRNA for excitatory G protein-coupled receptors was identified by ISH in the axon terminals of olfactory receptor neurons (42–44). Determining the location of GPR54 expression on GnRH neurons awaits the application of double-label IHC (with appropriate antibodies) and confocal microscopy. Some clues about this question may arise

Arcuate nucleus of the hypothalamus (ARC):

contains a distinct population of *Kiss1* neurons, GnRH neuronal fibers (and cell bodies in some species), and other neurotransmitters and neuropeptides; is associated with functions including the regulation of reproduction, body weight, and growth

Anteroventral periventricular nucleus (AVPV):

comprises a distinct population of *Kiss1* neurons

Medial preoptic area (MPOA): comprises cell bodies for GnRH neurons

P: progesterone

AR: androgen receptor

from the location of kisspeptin-containing fibers.

Anatomical evidence suggests that kisspeptin-containing neurons project to nuclei that comprise GnRH neurons. In the mouse, kisspeptin-containing fibers were described in the medial preoptic area (MPOA), the diagonal band of Broca (DBB), the ventral-lateral septum, and the anterior bed nucleus of the stria terminalis (BNST) (36). Clarkson & Herbison (36) showed that kisspeptin-containing fibers come in close contact with GnRH cell bodies and dendrites. The rostral preoptic area contains the greatest number of GnRH neurons with closely apposed kisspeptin fibers, with a smaller fraction in the DBB only in the female. The more rostral GnRH neurons in the medial septum do not appear to be near kisspeptin-containing fibers. Although researchers analyzed the close appositions between kisspeptin fibers and GnRH neurons with confocal microscopy (36), synaptic contacts between kisspeptin and GnRH neurons have not been confirmed by electron microscopy. Kisspeptin-containing fibers are also found in the ARC and the internal—but not external—zone of the ME (36, 41). Thus, at least in the mouse, kisspeptin is not likely to be secreted into the hypophysial portal vasculature as a hypophysiotropic hormone. However, in sheep, kisspeptin immunoreactive fibers were also described in the preoptic area and possibly the external zone of the ME (45). This result leaves open the possibility that kisspeptin in the sheep (and perhaps other species) is released into the portal circulation and thus influences pituitary hormone secretion. GPR54 is expressed in the pituitary (4, 46), and kisspeptin stimulates the release of LH and growth hormone from cultured rat pituitaries (46). These observations suggest that in some species either kisspeptins are delivered to the pituitary from the portal system or they act as autocrine signals between cells in the pituitary.

Kisspeptin activates GnRH neurons. Perforated patch-clamp recordings from GFP-

labeled GnRH neurons in slice preparations showed that kisspeptin excites GnRH neurons for long periods of time (47). Nanomolar concentrations of kisspeptin-10 depolarize and increase the firing rate of GnRH neurons in the adult male and female mouse. Depolarization continues for as long as the cells can be held (sometimes >60 min) and persists in the presence of tetrodotoxin (TTX), which indicates that kisspeptin-10 stimulates GnRH neurons directly. Molecular analysis of GnRH neurons after exposure to kisspeptin is consistent with the electrophysiological observations. ICV administration of kisspeptin induces Fos expression in GnRH neurons within 30 min of exposure (22, 23). Remarkably, Fos expression appears in *Kiss1* neurons in the AVPV concomitantly with Fos appearance in GnRH neurons on the afternoon of proestrus, when GnRH levels surge (28, 41). Thus, GnRH and *Kiss1* neurons become activated nearly simultaneously on the afternoon of proestrus.

A ROLE FOR KISSEPTINS AND GPR54 IN THE NEGATIVE FEEDBACK REGULATION OF GONADOTROPIN SECRETION BY SEX STEROIDS

Gonadal hormones provide a negative feedback signal to the brain-pituitary axis to maintain gonadotropin secretion within homeostatic boundaries, which supports normal gonadal function (i.e., hormone production and gametogenesis) in both sexes. Although the negative feedback effects of gonadal hormones occur at both the hypothalamus and pituitary (48, 49), here we focus on feedback regulation at hypothalamic sites. In the male, T acts as the inhibitory arm in a negative feedback loop that governs GnRH and LH pulse amplitude and frequency (50, 51); in the female E and progesterone (P) serve this same function throughout most of the estrous cycle (52). In the male, T appears to suppress gonadotropin secretion by activating both the androgen receptor (AR) and the

classical estrogen receptor (ER α), following a conversion of T to E by aromatase. Dihydrotestosterone (DHT), which binds AR but cannot be aromatized, and E can both suppress gonadotropin secretion (53). Furthermore, intact males with genetically targeted deletions of ER α (ER α KO) show only a modest increase in serum LH secretion (48). These findings suggest that ER- and AR-dependent mechanisms mediate the effects of T on gonadotropin secretion (at least in the mouse).

ER α also mediates the negative feedback effects of E in the female. Intact female ER α KO mice show elevated LH levels that do not change in response to ovariectomy (54, 55). In the female at certain times during the estrous cycle, P also contributes to the negative feedback control of gonadotropin secretion by acting through its own receptor, the progesterone receptor (PR). P reduces GnRH pulse frequency in the ewe (52), and PR KO mice display elevated levels of LH (56). Although androgens, E, and P suppress gonadotropin secretion through AR-, ER α -, and PR-dependent mechanisms (respectively), none of these sex steroids affect GnRH secretion by direct action on GnRH neurons. E is not concentrated in the nuclei of GnRH neurons (57), and neither AR (58, 59), ER α (60), nor PR (61–63) is found at significant levels in GnRH neurons. Although GnRH neurons do express estrogen receptor β (ER β) (64–66), this receptor does not appear to play an essential role in the regulation of GnRH secretion. In females, plasma levels of LH are almost indistinguishable between intact ER β KO and wild-type mice, and ER β KO mice respond to oophorectomy by increasing circulating levels of LH, just as their wild-type counterparts do (54, 55). Likewise, in the male, ER β does not appear to play an important role in the negative feedback control of gonadotropin secretion (48). Therefore, other sex steroid-responsive cells receive, transduce, and indirectly mediate the negative feedback effects of E, T, and P on GnRH neurons.

Studies in rat (67) and sheep (68) suggest that the mediobasal hypothalamus (MBH) and the ARC, in particular, are important sites for the negative feedback effects of sex steroids on gonadotropin secretion (67). ER α -expressing neurons in the ARC project to the rostral preoptic area (rPOA) in the rat (69) and project to the rPOA and DBB in the ewe (70)—areas where many of the cell bodies of GnRH neurons reside. In fact, viral tracing studies in the mouse have shown that GnRH neurons receive synaptic inputs from ER α -expressing neurons in the ARC (71). In the sheep, PR-containing neurons in the ARC project to the preoptic area (72, 73); in the rat, researchers have identified at least some AR-containing neurons in the ARC (74), which marks this region as one of the important target sites for the action of sex steroids on the brain.

The identity of neurons in the ARC upon which sex steroids act to regulate GnRH secretion remains to be fully elucidated. Recent evidence suggests that kisspeptin neurons may account for at least one population linking sex steroids to GnRH secretion. First, Navarro and coworkers (75) showed by RT-PCR that *Kiss1* mRNA in hypothalamic tissue fragments is negatively regulated by T in the male rat and by E in the female rat. Gonadectomy increases *Kiss1* mRNA levels, whereas replacement with gender-appropriate sex steroids reverses this effect (75). Second, steroid-dependent inhibition of *Kiss1* expression occurs specifically (and uniquely) in the ARC, as shown by ISH in the mouse (27, 35), Syrian hamster (29), ewe (31), macaque, and human (30) and by RT-PCR in the rat (76). In the male, the expression of *Kiss1* mRNA is regulated by the action of T through both the AR and the ER (35). DHT, a nonaromatizable androgen that binds to AR, and E, which binds to ER α , both can inhibit the increase in *Kiss1* mRNA in the ARC that occurs following castration. However, DHT only partially reverses the postcastration rise in *Kiss1* mRNA in the ARC, whereas E fully reverses it. This implies that the primary effect of T on *Kiss1*

Estrogen receptor α (ER α): the classical nuclear estrogen receptor

Dihydrotestosterone (DHT): binds the androgen receptor and cannot be converted to estrogen

PR: progesterone receptor

Estrogen receptor β (ER β): a nuclear estrogen receptor

Mediobasal hypothalamus (MBH): a caudal hypothalamic region that includes the arcuate nucleus

gene expression is mediated by ER α , presumably reflecting the conversion of T to E and the binding of E to ER α . Researchers see similar effects of DHT and E on LH secretion—DHT partially reverses the increase in LH that follows castration, whereas E fully reverses this increase (48, 53). Further supporting a role for both ER α and AR is that T regulates *Kiss1* expression in both ER α KO males and males with a hypomorphic allele of the AR (35). Sex steroids may act directly on kisspeptin neurons because, in the ARC of the male mouse, >85% of *Kiss1* neurons in the ARC express ER α and >60% express AR. The requirement of AR and ER α for mediating the feedback effects of sex steroids on *Kiss1* expression in the male remains to be confirmed by analysis of *Kiss1* regulation in AR and ER α double-KO animals.

As in the male, E inhibits *Kiss1* expression in the female, which occurs through ER α (27, 75). Navarro and colleagues (75) showed that either E or an ER α agonist [propylpyrazoletriol (PPT)] inhibits *Kiss1* mRNA expression (on the basis of RT-PCR measurements of the whole hypothalamus), whereas a potent selective ER β agonist [diarylpropionitrile (DPN)] does not. Smith and coworkers (27) corroborated and extended this observation by showing that the inhibitory effect of E on *Kiss1* expression occurs specifically in the ARC. The inhibitory effect of E on *Kiss1* expression in the ARC is abolished in ER α KO females but not in ER β KO females, which indicates that ER α , but not ER β , is required for the regulatory effects of E. As in the male, E probably acts directly on kisspeptin neurons in the female because nearly 100% of *Kiss1*-expressing neurons in the ARC express ER α mRNA and approximately 25% of these neurons coexpress ER β mRNA. E may act through ER β to regulate the expression of other genes expressed in *Kiss1* neurons, but the functional role of ER β in kisspeptin neurons remains to be established.

Similar observations of the regulation of *Kiss1* expression by E were reported for the

ewe. As in the mouse, ovariectomy in the ewe induces the expression of *Kiss1* mRNA in the ARC, and this effect is fully reversed by E replacement (31). E may also affect *Kiss1* expression in the ewe directly because nearly all kisspeptin immunoreactive neurons in the ARC also express ER α (45). Smith and coworkers (31) showed that P may also regulate *Kiss1* expression in the ewe. The administration of P to ewes partially reverses the increase in *Kiss1* mRNA that occurs following ovariectomy, and >85% of kisspeptin neurons in the ARC of the ewe express PR. Recent studies in humans also demonstrated that the expression of *Kiss1* mRNA in the ARC increases as a function of the reduction of sex steroid production associated with menopause (30). A similar phenomenon occurs in the female macaque following ovariectomy (30), which suggests that *Kiss1* neurons in the ARC of the primate—as in the case of the rodent—are targets for the inhibitory action of sex steroids and thus may mediate the negative feedback effects of sex steroids on GnRH secretion.

Researchers have also examined the regulation of the kisspeptin receptor, GPR54, by sex steroids, although it is difficult to draw clear conclusions. First, via the use of real-time RT-PCR on the rat hypothalamus, Navarro and coworkers (75) found that *GPR54* mRNA is inhibited by T in the male and by E in the female. As with *Kiss1*, the regulation of *GPR54* in the female rat depends on the activation of ER α , but not that of ER β . In the adult male monkey, sex steroids do not regulate the expression of GPR54 (on the basis of RNase protection assays of the MBH) (77). It is important to recognize that *GPR54* mRNA is expressed in various regions of the forebrain, and measurements of hypothalamic content by RT-PCR may obscure discrete regional changes that occur as a function of the steroidal milieu. Further studies of GPR54 in specific cell types are required to clarify the precise effect of sex steroids on the expression of *GPR54* in the brain, particularly

as it relates to the regulation of reproductive function.

The expression of *Kiss1* in the forebrain changes as a function of the estrous cycle in the rat—again, perhaps as a reflection of the role of kisspeptin-GPR54 signaling in the negative feedback regulation of gonadotropin secretion throughout most of the estrous cycle. RT-PCR measurements of total hypothalamic content demonstrate that *Kiss1* mRNA levels are highest on diestrus 1, when circulating levels of sex steroids are low (75). Conversely, during estrus, when E and P levels are declining from their peak levels during proestrus, the expression of *Kiss1* is at its lowest ebb. Further studies have shown that total hypothalamic alterations in the expression of *Kiss1* largely reflect changes that occur specifically in the ARC, which contains an abundance of *Kiss1* mRNA (28, 76). These findings are consistent with a model in which kisspeptin neurons in the ARC may provide tonic—but variable—stimulatory input to GnRH neurons. In this model, the activity of *Kiss1* neurons in the ARC diminishes when sex steroid levels rise and increases when sex steroids levels fall, thus gating the operation of GnRH neurons in a classical negative feedback loop (Figure 2). In support of this model is the observation that mice with genetically targeted deletions in the *GPR54* gene lack the ability to show a compensatory rise in LH secretion following gonadectomy—despite a marked increase in *Kiss1* expression in the ARC (78)—which suggests that kisspeptin-GPR54 signaling is essential for negative feedback regulation of gonadotropin secretion. Recent studies in the macaque and sheep indicate that *Kiss1* neurons in the ARC may play a similar role in the negative feedback control of gonadotropin secretion in these species as well (31, 77). Nevertheless, evidence to support the negative feedback model remains circumstantial, and further studies with targeted deletions of *Kiss1* expression in the ARC (or ER α signaling in *Kiss1* neurons in the ARC) are necessary to establish the model's validity.

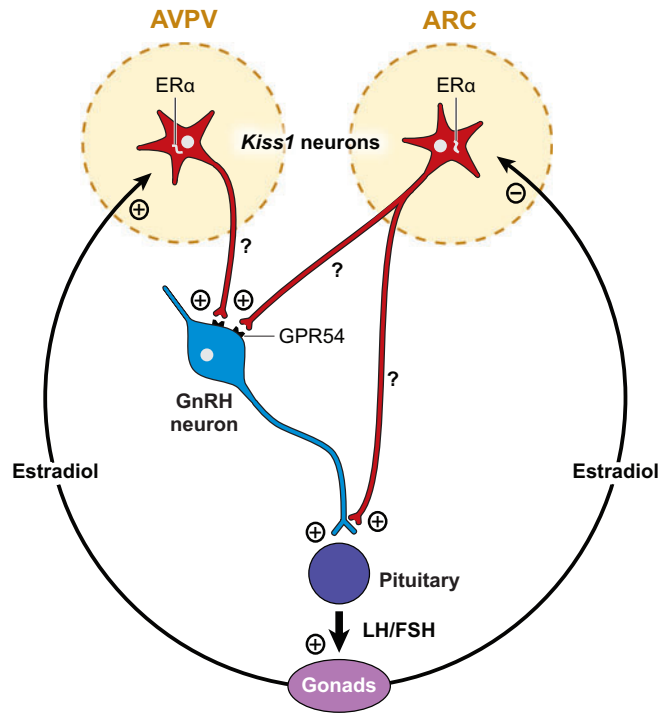


Figure 2

Kiss1-expressing neurons may relay negative and positive feedback effects of sex steroids on gonadotropin-releasing hormone (GnRH) secretion. Kisspeptin excites GnRH neurons and stimulates GnRH release by acting on the G protein-coupled receptor GPR54. GnRH release results in increased gonadotropin secretion from the pituitary gland, which stimulates sex steroid synthesis and secretion from the gonads. Sex steroids (e.g., estrogen) regulate *Kiss1* expression, such that inhibition of *Kiss1* in the arcuate nucleus of the hypothalamus (ARC) may reduce kisspeptin input to GnRH neurons and reduce GnRH and gonadotropin release in both sexes. In females, stimulation of *Kiss1* expression by estrogen in the anteroventral periventricular nucleus (AVPV) may increase kisspeptin input to GnRH neurons as well as GnRH and gonadotropin release. Other abbreviations used: AVPV, anteroventral periventricular nucleus; ER α , estrogen receptor α ; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

A ROLE FOR KISSPEPTINS AND GPR54 IN THE POSITIVE FEEDBACK REGULATION OF GnRH/LH SECRETION BY ESTROGEN

The negative feedback of gonadal steroids on GnRH secretion is a common feature of males and females, whereas the positive feedback effects of E and P that drive the preovulatory surge in GnRH are sexually differentiated and occur only in females (79–81). In the

female rat, beginning in diestrus II and during proestrus, the rising tide of E acts initially to inhibit gonadotropin secretion (i.e., negative feedback). However, the effect of E on LH secretion suddenly reverses on the late afternoon of proestrus to unleash a torrent of GnRH secretion, which in turn stimulates LH secretion and triggers ovulation. Sexual differentiation of the E-induced GnRH/LH surge mechanism is established during early postnatal development (in the rat and mouse). In the first week of postnatal life, during the so-called critical period, normal males experience relatively high levels of circulating T, which permanently ablates their ability to elicit an LH surge in response to E (82–84). Normal females, who are not exposed to T during the critical period, develop the capacity to elicit a GnRH/LH surge in response to E. However, females that are experimentally exposed to T during the critical period behave like males as adults—i.e., they lack the capability to demonstrate an E-induced LH surge, whereas males that are castrated early in postnatal life (and are not exposed to T) behave like females—i.e., they retain the capacity to demonstrate an E-induced LH surge (83, 84). However, in higher primates, males retain the ability to generate an LH surge in response to E, at least in some circumstances (85–87).

In the rodent, neuronal signals that trigger the preovulatory GnRH/LH surge converge in the AVPV (67, 80). Lesions of the AVPV disrupt spontaneous estrous cycles and the E/P-induced LH surge (88, 89), whereas E placed directly into the AVPV evokes an LH surge (90). The AVPV is sexually differentiated and contains more steroid-responsive neurons in the female than in the male (91). Certain populations of neurons in the AVPV, including dopaminergic neurons, are sexually differentiated and more abundant in the female (92). The expression of *Kiss1* in the AVPV is also sexually differentiated—at least in the rat and mouse (34, 36, 76): Females express more *Kiss1* mRNA and kisspeptin than do males. In the rat, the population of *Kiss1* neurons in the AVPV is distinct from the sex-

ually differentiated population of dopaminergic neurons (34). However, many *Kiss1* neurons in the mouse AVPV coexpress tyrosine hydroxylase (TH) and are thus dopaminergic (93). Not only do females have more kisspeptin neurons in the AVPV than do males, but they also possess more kisspeptin fibers in close apposition to GnRH neurons (36). Sexual differentiation of *Kiss1* in the AVPV depends on the organizational effects of circulating sex steroids during prepubertal development, evidenced by the fact that treatment of prepubertal females with T reduces the level of *Kiss1* expression in the AVPV (but not the ARC) of the adult (34). GPR54 signaling may also be sexually differentiated because depolarization of GnRH neurons by kisspeptin is always associated with firing in females, but not in males (47). However, the basis for sexual differentiation of the kisspeptin-GPR54 signaling mechanism remains unclear (47).

ER α mediates the positive feedback effects of E on GnRH/LH secretion (94). Female mice with either global or neuronal-specific deletions in the *ER α* gene are incapable of producing an LH surge in response to E (71). Evidence suggests that a population of ER α -responsive cells in the AVPV drives the preovulatory GnRH surge. In the rat, ER α -expressing neurons from the AVPV project to the rPOA, where GnRH neurons reside (69). In the mouse, GnRH neurons receive direct afferent input from ER α -expressing neurons whose cell bodies reside in the AVPV (71). The PR also plays an important role in the stimulation of the preovulatory LH surge. Female PR KO mice do not display E-induced LH surges (95), which indicates that some effects of E lead to the activation of PR. Furthermore, E upregulates PR expression (96). Thus, signaling through ER α -dependent mechanisms in the AVPV appears to be a prerequisite for the generation of the preovulatory GnRH/LH surge in rodents.

Although *Kiss1* neurons in the ARC are implicated in the negative feedback regulation of GnRH/LH secretion, *Kiss1* neurons in

the AVPV are compelling candidates for the generation of the positive feedback effects of E (**Figure 2**) (27, 28, 35, 76). In the female AVPV, E dramatically induces the expression of *Kiss1* mRNA (27, 28, 76), ER α mediates the positive feedback of E on *Kiss1*, and virtually all *Kiss1* neurons in the AVPV express ER α (27). On the afternoon of proestrus, there is a robust increase in the expression of *Kiss1* mRNA as well as an increase in the number of Fos-expressing *Kiss1* cells in the AVPV (28, 41, 76). In addition, Fos induction in *Kiss1* neurons of the AVPV occurs concurrently with Fos induction in GnRH neurons on the afternoon of proestrus in the rat (28, 97). Although it seems clear that *Kiss1* neurons in the AVPV are activated at the time of the GnRH/LH surge in the rat, there are conflicting reports about whether *Kiss1* neurons in the ARC also express Fos at the same time (41). There is an ongoing debate about the specificity of many of the kisspeptin antibodies used for IHC (28), which may contribute to the controversy. Perhaps this issue will be resolved as better kisspeptin antibodies become available. In any case, central kisspeptin activity may be essential for positive feedback because central infusion of kisspeptin antibody blocks the LH surge and estrous cyclicity (41, 76).

These findings suggest that kisspeptin activity is essential for generating GnRH/LH surges; however, for several reasons these findings do not prove that kisspeptin regulation by E in the AVPV is solely responsible for driving the endogenous preovulatory GnRH/LH surge. First, kisspeptin-induced LH release in female rats is enhanced when both E and P are present (i.e., during proestrus, estrus, OVX + E and P) compared with times when P is low or absent (i.e., diestrus 1 and 2, OVX + E), which suggests that increased responsiveness of the GPR54/GnRH/LH pathway to P may also be an important component in the generation of GnRH/LH surges (98). Second, other periventricular regions that express *Kiss1* may also be involved in positive feedback. *Kiss1*

is expressed in the periventricular nucleus (PeN), which is contiguous with the AVPV, and *Kiss1* neurons in this region respond to sex steroids similarly to *Kiss1* neurons in the AVPV (27). This nucleus also contains ER α -expressing neurons that project to GnRH neurons (71), which may include *Kiss1* neurons (27). Finally, recent evidence suggests that mice with genetically targeted deletions in *GPR54* retain the capacity to produce a GnRH/LH surge in response to E (78). Thus, the relative contribution of kisspeptin-GPR54 signaling in the generation of the GnRH/LH surge remains to be elucidated fully.

There are relatively few *Kiss1* neurons in the male AVPV, unlike the female (34, 99). As in the female, sex steroids (E and T) can stimulate *Kiss1* gene expression in the AVPV of the male (35). Although *Kiss1* neurons in the female have been implicated in the GnRH/LH surge mechanism, this process is not relevant in the male, which lacks this capacity. The few *Kiss1* neurons that are present (and regulated) in the male may simply reflect vestigial elements that are retained despite apoptosis within the AVPV during the neonatal critical period (80, 100). Conversely, *Kiss1* neurons in the AVPV may be involved in other T-dependent processes, such as sexual behavior (35). Recent studies of the *GPR54* KO mouse by Kauffman and colleagues (99) have shown that kisspeptin-GPR54 signaling is required for sexual differentiation of *Kiss1* expression in the AVPV (as well as other sexually differentiated populations of neurons in the brain and spinal cord) and for certain gender-specific behaviors, such as partner preference, but not for either male or female copulatory behaviors.

In certain species, including sheep, the ARC has been implicated in both the negative and positive feedback control of gonadotropin (101, 102). Consistent with such a dual role, *Kiss1* expression can be induced (31, 103) and suppressed (31) by the administration of E under different treatment regimens. Whether the opposing effects of E on *Kiss1* expression

occur in different subregions of the ARC or in phenotypically unique groups of cells that lack regional organization remains to be determined.

The role—if any—of *Kiss1* neurons in the generation of the GnRH/LH surge in the primate remains unexplored. Controversy remains about the relative role of GnRH neurons in the rostral forebrain versus the MBH for the generation of the preovulatory LH surge in the primate (105–107). In the monkey and in humans, *Kiss1*-expressing neurons are concentrated in the infundibular nucleus (30) (the homolog of the ARC), which is reminiscent of the distribution of *Kiss1* neurons in the sheep (31, 103). Whether a discrete population of *Kiss1* neurons in the infundibular nucleus of the female primate plays a role in the generation of the preovulatory GnRH/LH surge remains to be explored.

KISSPEPTIN-GPR54 SIGNALING AND THE INITIATION OF PUBERTY

Puberty in the Primate

Kisspeptin-GPR54 signaling is a prerequisite for normal pubertal development in the primate. The first evidence came from studies of humans with IHH. Humans with homozygous mutations in *GPR54* do not attain normal puberty (13, 14). Since the initial discovery of the role of GPR54 in reproduction in 2003, researchers have described similar cases of patients with *GPR54* mutations (108, 109). In one study (109), researchers reported small LH pulses in a female IHH patient with a homozygous *GPR54* mutation. The authors propose that if this mutation (L102P) completely abolishes GPR54 signaling, GPR54 activation only enhances the amplitude of GnRH pulses at puberty, rather than playing an obligatory role in triggering the pulses. One may imagine that kisspeptin acts as a neuromodulator, enhancing excitatory input from other neurotransmitters, such as glutamate. The aforementioned study also noted

that the L102P mutation disrupts PI accumulation (a measure of PLC activity) in HEK293 cells; however, whether this mutation would fully disrupt GPR54 function in GnRH neurons and whether GPR54 activation plays a quantitative or qualitative role in the stimulation of GnRH secretion at puberty remain undetermined.

Studies in the nonhuman primate also suggest that kisspeptins and GPR54 contribute to the onset of puberty. Central or peripheral kisspeptin administration leads to precocious release of GnRH and LH in the juvenile male monkey (24). Intermittent kisspeptin administration is more effective at maintaining GnRH secretion in these animals (25, 26) than is continuous kisspeptin infusion, which produces desensitization of the GPR54 receptor. These results suggest that endogenous kisspeptin activity may also be pulsatile and that enhanced, pulsatile kisspeptin secretion may drive increased GnRH release at puberty. Consistent with this hypothesis, hypothalamic levels of *Kiss1* mRNA increase across pubertal development in both the male and female monkey (24). Hypothalamic expression of *GPR54* also increases in the female monkey at puberty (although apparently not in agonadal males) (24), which suggests that the GnRH neuronal network becomes more sensitive to kisspeptin's effects as a function of development; however, it remains to be determined whether the increase in GPR54 occurs in GnRH neurons or in other cell populations that modulate GnRH secretion.

Puberty in the Rodent

The neuroendocrine mechanisms that control the onset of puberty in rodent species may be fundamentally different from those operating in the primate (110, 111); however, kisspeptin signaling through GPR54 may be the pivotal event governing the onset of puberty in the rodent, as is the case in primate species. Mice with genetically targeted deletions in *GPR54* exhibit a similar phenotype as humans with disabling mutations in *GPR54*—they do not

progress through normal sexual maturation (14, 16). Kisspeptin induces LH and FSH secretion in the prepubertal male and female rat and in the prepubertal male mouse. However, kisspeptin may be less potent in stimulating LH in the prepubertal animal compared with the adult, which suggests that this pathway may not be fully engaged before puberty (47, 75, 112). Nevertheless, chronic ICV kisspeptin administration [from postnatal day (P)26 to P31] induces precocious puberty in the female rat, which is reflected by increased sex hormone production, increased uterine weight, and early vaginal opening (113).

Kisspeptin's stimulatory effect on pubertal maturation appears to be downstream of leptin and perhaps other peripheral signals of nutritional state. Kisspeptin stimulates LH (113, 114) and FSH (112) secretion in leptin- and insulin-resistant (*fa/fa*) rats, food-restricted pubertal and prepubertal female rats, and immature female rats treated with an anti-leptin antibody (112–114). Kisspeptin also rescues fasting-induced deficits in the prepubertal rat; these include delayed vaginal opening, decreased GnRH secretion, and low serum levels of LH and E (114). Thus, kisspeptin may be one of the more proximate inputs to GnRH neurons.

Kisspeptin stimulation of GPR54 likely increases around the time of puberty. In the rat, maximal expression of hypothalamic *Kiss1* and *GPR54* mRNA, as measured by RT-PCR, occurs at puberty (75). *Kiss1* mRNA also increases between the juvenile and adult state in the AVPV and PeN of the male mouse—but not in the ARC (47). The number of kisspeptin cells (as determined by IHC) in the AVPV/PeN of the male and female mouse also increases across pubertal development and is accompanied by an increase in the number of kisspeptin-containing fibers, which has been reported only in the female (36). Upregulation of kisspeptin may enhance GnRH secretion because the number of kisspeptin-containing fibers that come in close apposition with GnRH neurons increases throughout postnatal development (36). In the female,

upregulation of *Kiss1* expression in the AVPV/PeN may explain the increased ability of the rat to respond to the positive feedback effects of E on gonadotropin secretion as the animal matures. However, whether the increase in *Kiss1* expression in the AVPV/PeN that occurs at the onset of puberty is steroid dependent or steroid independent remains undetermined. If the increase in *Kiss1* expression is steroid dependent, it may not trigger the onset of puberty but rather may only reflect the physiological consequences of puberty.

In addition to the putative increase in kisspeptin input to GnRH neurons that may occur as a function of pubertal development, GnRH neurons may also become more sensitive to kisspeptin stimulation. Perforated patch-clamp recordings from GnRH neurons in slice preparations show an increased responsiveness to kisspeptin as a function of pubertal development (47). GnRH neurons from juvenile animals are initially depolarized by kisspeptin, but their response is transient, lasting only 2–3 min. In contrast, GnRH neurons from the adult mouse have a prolonged depolarization, typically lasting as long as the recording (usually 30 min). In vivo results corroborate these in vitro observations. In experiments with ICV injections of kisspeptin, only the highest dose of kisspeptin tested (0.1 nmol) induced an LH response in juvenile mice, whereas even the smallest dose tested (10 fmol) increased serum LH levels in adult mice (47). Furthermore, kisspeptin's effect on LH secretion in the adult male rat is more prolonged than in the prepubertal male rat (75). The apparent increase in sensitivity of GnRH neurons to kisspeptin is likely not the result of an increase in *GPR54* mRNA in GnRH neurons of the mouse, as shown by double-label ISH (47). The enhanced sensitivity to kisspeptin may be attributable to post-transcriptional regulation of GPR54 such as translation or insertion in the plasma membrane. Enhancement of the GPR54 signaling cascade may also explain the increased sensitivity to kisspeptin.

Despite the evidence that *Kiss1*/kisspeptin-GPR54 signaling plays a critical role in the onset of puberty in primates and rodents, many questions remain. For instance, it is unclear whether kisspeptin-GPR54 signaling is a triggering event for pubertal maturation or just one of the myriad factors that are simply permissive (necessary but not sufficient)—reminiscent of the role of leptin or insulin in pubertal maturation (115–118). Most likely, kisspeptins and GPR54 do not act alone to initiate puberty but rather are permissive elements in a complex network of transmitters and hormones that guide the trajectory of pubertal maturation.

KISSPEPTIN-GPR54 SIGNALING: A POSSIBLE LINK BETWEEN METABOLISM AND REPRODUCTION

Reproduction is energetically demanding. In animals, a negative energy balance (due to, e.g., starvation, excessive exercise, or lactation) inhibits the reproductive axis. This inhibition may take the form of either a delay in pubertal onset or an interruption of normal function in the adult (119). Nutritional status is communicated to the neuroendocrine reproductive axis by circulating factors that include metabolic fuels (e.g., glucose) and hormones produced by peripheral tissues (e.g., leptin and insulin). Although metabolic fuels and hormones can affect all three components of the hypothalamic-pituitary-gonadal (HPG) axis, their effects are mediated primarily by the brain (119).

Researchers have recently investigated the possibility that kisspeptin neurons relay metabolic information to the reproductive axis. If kisspeptin neurons do serve this function, *Kiss1* expression might be regulated by fasting or metabolic hormones, such as leptin or insulin. Indeed, fasting decreases hypothalamic *Kiss1* mRNA levels in prepubertal male and female rats, as shown by RT-PCR (114). Because kisspeptin stimulates GnRH secretion, it seems plausible that some effects of

fasting on the reproductive axis are mediated by a reduction of kisspeptin synthesis and secretion. Fasting increases hypothalamic levels of *GPR54* mRNA, which may explain the apparent increased sensitivity of the HPG axis to kisspeptin in fasted animals (114). This observation is consistent with a role for kisspeptin in mediating some of leptin's effects on the HPG axis. Fasted animals show reduced circulating levels of leptin; leptin, like kisspeptin, stimulates LH secretion more in malnourished animals than in well-fed animals (119). Furthermore, leptin-deficient (*ob/ob*) mice show decreased *Kiss1* mRNA levels in the ARC relative to their wild-type counterparts, and leptin partially restores *Kiss1* expression in the ARC of the *ob/ob* mouse (120). However, that *Kiss1* expression is not fully restored by leptin administration in these animals suggests that other defects in the *ob/ob* mouse that are not restored by leptin treatment (such as elevated corticosterone and thyroid hormone or poor insulin signaling) may also account for some reduction of *Kiss1* expression. Nevertheless, leptin seems to regulate the *Kiss1* gene in the mouse directly because 40% of *Kiss1*-expressing neurons in the ARC express mRNA for the active form of the leptin receptor (*Ob-Rb*) (120). In this study, researchers failed to detect differences in *Kiss1* expression in the AVPV between wild-type and *ob/ob* male mice. However, castrated male animals were used in these experiments, and relatively few *Kiss1* neurons could be detected in the AVPV under these circumstances (27, 35). In any case, *Kiss1* neurons in the ARC are clearly targets for the action of leptin (**Figure 3**).

The role of kisspeptin in mediating the effects of metabolic signals on the HPG axis has also been studied in the streptozotocin (STZ)-treated diabetic rat. These diabetic rats exhibit decreased levels of hypothalamic *Kiss1* mRNA, as measured by RT-PCR (121). Decreased *Kiss1* expression (and, by inference, decreased excitatory input to GnRH neurons) in these animals may explain their low circulating levels of gonadotropin and sex steroids. Consistent with this hypothesis,

kisspeptin administration restores serum LH and T levels to normal and partially restores prostate and testicular weight in these animals (121). Furthermore, the rise in both LH levels and hypothalamic *Kiss1* expression in response to castration is blunted in diabetic rats compared with controls. Kisspeptin treatment of diabetic rats restores LH levels to those of control animals (121). However, elevated gonadotropin levels in response to exogenous kisspeptin do not prove that kisspeptin neurons compose the conduit that relays metabolic signaling to the neuroendocrine reproductive axis. Nevertheless, if kisspeptin neurons do serve this function, it would be fascinating to identify which peripheral factors are responsible for decreased *Kiss1* expression in diabetic animals. It may be noteworthy that in these animals, leptin, but not insulin, restores hypothalamic *Kiss1* mRNA levels to those of control animals (121), restores LH and T levels, and partially restores prostate and testicular weight (121). These findings provide further circumstantial evidence that kisspeptin mediates some of leptin's effects on reproduction. Thus far, the results are consistent with a model whereby leptin and perhaps other adiposity and satiety factors stimulate *Kiss1* expression, which allows for the adequate release of kisspeptin and trophic stimulation of GnRH release. When levels of adiposity and satiety factors decrease or when such factors are not detected, the expression of *Kiss1* (and presumably its secretion) decreases, thus reducing excitatory input to GnRH neurons.

Lactation is a state of negative energy balance—much like continuous exercise. During lactation, ovulation and folliculogenesis are suppressed (122), and estrous cycles are inhibited as a result of decreased GnRH/LH secretion (123). The central mechanisms that inhibit GnRH secretion during lactation remain mysterious; however, recent evidence suggests that *Kiss1* neurons may play a pivotal role in this process. Indeed, lactating rats have lower levels of *Kiss1* mRNA and kisspeptin protein in the ARC compared with nonlac-

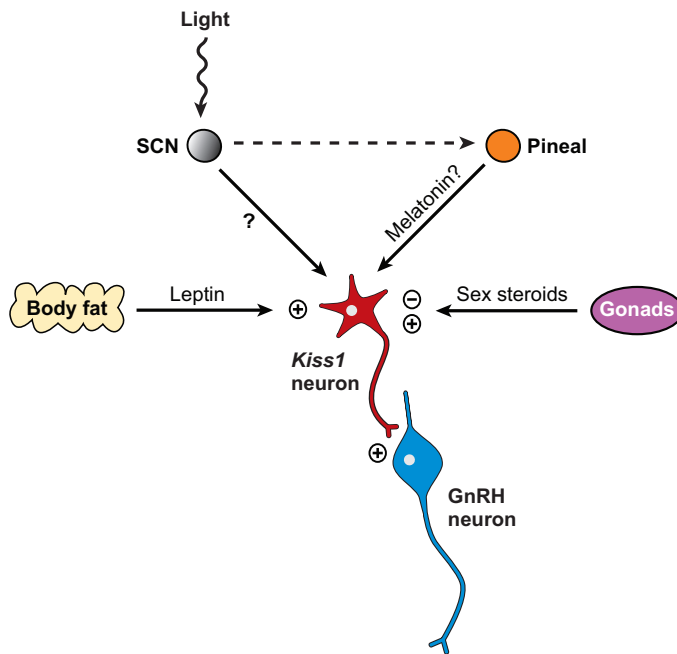


Figure 3

Kiss1 neurons as conduits for the modulation of gonadotropin-releasing hormone (GnRH) secretion by environmental factors. A variety of internal and external factors regulate *Kiss1* expression, which may modulate the activity of GnRH neurons and the reproductive axis. The dashed arrow represents the indirect projection from the suprachiasmatic nucleus (SCN) to the pineal gland.

tating controls (124). Thus, a reduction of tonic kisspeptin drive to the HPG axis may occur during lactation, a time when pregnancy would be disadvantageous. Furthermore, although kisspeptin stimulates LH release in lactating rats (98, 124), there is some evidence that the brain-pituitary axis is less sensitive to kisspeptin during lactation (98). This apparent desensitization may be explained by the decrease in the expression of *GPR54* mRNA in the AVPV of lactating rats (124), although this process remains to be fully elucidated.

Kisspeptin may mediate the permissive role of metabolic factors in the onset of puberty. Studies in prepubertal or pubertal female rodents show that kisspeptin can override reproductive deficiencies triggered by metabolic disturbances (112–114). This suggests that kisspeptin signaling lies downstream of the metabolic factors that regulate

Suprachiasmatic nucleus of the hypothalamus

(SCN): receives and communicates circadian light cues

reproduction; however, any possible role of kisspeptin in the control of the tempo of pubertal maturation, influenced by nutrition and metabolic reserves, remains to be established.

Hormones such as leptin and insulin influence metabolism and reproduction, feeding, body weight, and gonadotropin secretion (119). Current evidence suggests that unlike leptin and insulin, kisspeptin does not play a direct role in the regulation of either metabolism or body weight. The exogenous administration of kisspeptin does not alter food intake (114); moreover, humans or mice with mutations in *GPR54* do not show disruptions in metabolism or body weight (14). However, kisspeptin may have subtle effects on metabolism, which are yet to be discovered.

LIGHT MAY REGULATE THE REPRODUCTIVE AXIS BY ACTING ON KISSPEPTIN NEURONS

Most mammals that live beyond the tropical equatorial latitudes show seasonal changes in their ability to reproduce. Photoperiod is usually the most reliable indicator of seasonal change to the reproductive axis. Therefore, seasonal breeders not only entrain their daily rhythm to the light-dark cycle but also rely on changes in day length throughout the year to reproduce at the most opportune time (125). Seasonal control of reproduction has been studied most extensively in Syrian and Siberian hamsters and in sheep (125). Hamsters reproduce as days become longer and enter reproductive quiescence as days become shorter (125). In contrast to hamsters, sheep become reproductively active as days become shorter, and enter anestrus (reproductive quiescence) as days become longer (125–128). Evidence suggests that photoperiodic information is transmitted to the reproductive system by melatonin, a hormone synthesized by the pineal gland. The suprachiasmatic nucleus of the hypothalamus (SCN) receives information about light from the retina and stimulates

melatonin synthesis at night through a series of synaptic connections (**Figure 3**) (125). Studies in which melatonin is administered to mimic different photoperiods demonstrate that day length is encoded by the duration of melatonin secretion in both hamsters and sheep (125).

Recent evidence suggests that the expression of *Kiss1* is regulated by photoperiod and may function as a gateway to seasonal awakening of the neuroendocrine reproductive axis. *Kiss1* mRNA and kisspeptin are expressed in the ARC of seasonal breeders, including the Syrian and Siberian hamsters (29, 38) and the ewe (31). In male Syrian hamsters, short days (SD) inhibit the expression of *Kiss1* in the ARC (29). Reduction of *Kiss1* expression correlates with inhibition of the reproductive state because both long day (LD)- and SD-refractory animals (whose reproductive systems reactivate in spite of continued SD conditions) have higher testes weight and greater levels of *Kiss1* expression compared with SD animals (29). The apparent photoperiodic regulation of *Kiss1* is likely not caused by indirect effects of T in Syrian hamsters because T implants do not affect *Kiss1* mRNA levels in SD animals (29). Furthermore, gonadal steroids inhibit *Kiss1* mRNA in the ARC of the LD- and SD-refractory Syrian hamster (29). Thus, if endogenous T did mediate the photoperiodic effects, its reduction in SD animals would likely upregulate, not downregulate, *Kiss1* expression. These findings suggest that SD inhibits the activity of *Kiss1* neurons in the ARC of the Syrian hamster, which may decrease tonic stimulatory drive to GnRH neurons. The effects of photoperiod on *Kiss1* expression may be mediated by melatonin in the Syrian hamster. Pineal ablation prevents downregulation of *Kiss1* mRNA and reproductive inactivation in SD animals (29). The difference in *Kiss1* expression between pinealectomized and intact SD animals is quantitatively similar to that between intact SD and LD hamsters (29). Whether melatonin removal is responsible for the effects of pinealectomy remains to be

confirmed by melatonin replacement. If melatonin does regulate *Kiss1*, it is important to determine whether melatonin acts directly on kisspeptin neurons, through intermediate targets, or both (**Figure 3**).

The expression of kisspeptin is also regulated by photoperiod in Siberian hamsters. In male Siberian hamsters, SD is associated with an increase in kisspeptin levels (measured by IHC) in the ARC and a decrease in kisspeptin levels in the AVPV (38). The findings in the ARC of the Siberian hamster are opposite of those described in the Syrian hamster, in which SD decreases the levels of *Kiss1* mRNA and kisspeptin (29). This discrepancy may reflect a true species difference in photoperiodic regulation of kisspeptin, the use of different kisspeptin antibodies, or the use of different time points in the analyses. The authors suggest that an increase in kisspeptin in the ARC of the Siberian hamster may reflect a reduction of kisspeptin release and an accumulation of the peptide. However, a similar increase in kisspeptin is not seen in the Syrian hamster. Whether photoperiod regulates kisspeptin release and synthesis differently in the two species remains unresolved. In the Siberian hamster, SD has similar effects on *Kiss1* expression in the ARC and AVPV as does castration of other rodents (27, 28, 35, 76). Thus, the reported changes in kisspeptin expression may result from a reduction of circulating androgens during SD—this possibility remains to be tested in both Syrian and Siberian hamsters by exposing castrated animals to SD and LD conditions.

Although kisspeptin expression may be regulated differently by photoperiod in Syrian and Siberian hamsters, kisspeptin stimulates the HPG axis in SD animals of both species. Chronic ICV administration of kisspeptin restores testicular weight and T levels of SD hamsters to levels found in animals that are returned to LD (29). Because of the ICV administration of kisspeptin in this experiment, the effect is likely attributable to the stimulation of GnRH. Peripheral kisspeptin injection stimulates LH secretion in LD and

SD Siberian hamsters (38). These findings demonstrate that kisspeptin expression is regulated by photoperiod and is sufficient to restore HPG activity in seasonal breeders during the nonreproductive season. However, these findings do not prove that kisspeptin expression is necessary, nor do they exclude the involvement of other neurotransmitters in this process. Importantly, *Kiss1* expression is not regulated by photoperiod in the Wistar rat, which is not a seasonal breeder (29). This suggests that photoperiodic regulation of *Kiss1* is an important aspect of the neuroendocrine regulation of reproduction in seasonally breeding animals. Moreover, studies conducted to date in the hamster have involved only males. Further investigations in both sexes are necessary. That *Kiss1* is regulated by photoperiod and perhaps by melatonin makes kisspeptin neurons good candidates for relaying photoperiodic signals to the reproductive axis.

Seasonal regulation of *Kiss1* expression has also been reported in the ewe. As in the Syrian hamster, *Kiss1* mRNA levels in the ARC of the sheep are reduced during the nonbreeding season (compared with the breeding season) (31). Because this study was conducted in OVX animals, these results suggest that the seasonal regulation of *Kiss1* in the ewe is steroid independent. Whether the reduction of *Kiss1* in the ewe is due to photoperiod or other factors, such as temperature or metabolic cues, is unknown. If photoperiod is responsible for the seasonal change in *Kiss1*, it is important to figure out whether melatonin mediates this effect. It is remarkable—and mysterious—that the *Kiss1* gene is upregulated in breeding conditions in both LD and SD breeders. Reproductive awakening during the breeding season may have similar mechanisms as those that control the onset of puberty. Smith and coworkers (31) postulate that in addition to increased *Kiss1* expression, kisspeptin synaptic input to GnRH neurons may increase as animals approach the breeding season. This possibility remains to be explored.

CONCLUSION

In recent years, the essential role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction has become evident. Kisspeptins stimulate GnRH secretion directly, and *Kiss1* expression in the brain is correlated positively with conditions known

to stimulate the reproductive axis. However, GnRH neurons receive inputs from other cell types in the brain, which in turn receive thousands of inputs from other cells. It is important to learn how GnRH and *Kiss1* neurons integrate signals from this complex network to regulate gonadotropin secretion.

SUMMARY POINTS

1. Kisspeptins are products of the *Kiss1* gene that bind the G protein–coupled receptor GPR54.
2. GPR54 is necessary for normal puberty.
3. Kisspeptins directly stimulate GnRH secretion.
4. Gonadal sex steroids stimulate or inhibit *Kiss1* gene expression in hypothalamic neurons, depending on their location.
5. *Kiss1* neurons in the ARC are implicated in the sex steroid–dependent negative feedback control of gonadotropin secretion, whereas *Kiss1* neurons in the AVPV may be involved in generating the preovulatory GnRH/LH surge.
6. Pubertal onset may be triggered by increases in *Kiss1* expression, kisspeptin input to GnRH neurons, and GnRH neuron sensitivity to kisspeptins.
7. *Kiss1* neurons may relay metabolic signals to the reproductive axis, as *Kiss1* is positively regulated by metabolic factors and kisspeptin reverses reproductive defects seen in diabetic animals.
8. *Kiss1* neurons may mediate seasonal effects of light on reproduction, as *Kiss1* expression is regulated by photoperiod and season in seasonally breeding animals.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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