The Role of Kisspeptin Signaling in Reproduction

Kisspeptins are a group of peptides that stimulate GnRH release and are required for puberty and maintenance of normal reproductive function. This review focuses on our understanding of the way in which kisspeptin signaling regulates mammalian fertility and how they act as central integrators of different hormonal and physiological signals.

Acquisition of reproductive competency is essential for continuation of all species. Fertility in mammals is initiated at puberty by the pulsatile secretion of gonadotrophin releasing hormone (GnRH) from a small number of neurons in the hypothalamus (FIGURE 1). The GnRH is released into the hypophyseal portal blood system from nerve terminals in the palisade layer of the median eminence of the hypothalamus. The GnRH acts on the anterior pituitary to stimulate the release of the gonadotropic hormones, luteinizing hormone (LH), and follicle stimulating hormone (FSH). The gonadotropic hormones act on the gonads to stimulate sexual maturation and gametogenesis (spermatogenesis in males and oogenesis in females). The gonads produce sex steroids (testosterone in males and estrogen and progesterone in females), which are required for gametogenesis, for maturation of accessory sex organs, and to provide hormonal feedback loops that regulate GnRH and gonadotrophic hormone release under different physiological conditions (FIGURE 1). Although GnRH neurons are a critical component of the reproductive axis, kisspeptin (Kp) peptides have been identified recently as vital upstream regulators that integrate central and peripheral signals with GnRH release, thereby playing a pivotal role in the control of reproduction (FIGURE 1).

Kisspeptin Expression Profile

Kisspeptins are an overlapping set of amidated peptides encoded by the Kiss1 gene, which is located in close proximity to the Golt1a (golgi transport 1 homolog A) gene in most species. In humans and mice, the Kiss1 gene consists of two coding exons downstream from at least one noncoding exon (60) (FIGURE 2). The human promoter has been mapped immediately upstream of the non-coding exon and contains a TATA box and several potential SP1 transcription factor binding sites. These SP1 binding sites facilitate binding of AP-1s/SP1 and DRIP130/SP1 transcriptional activator complexes and contribute to basal promoter activity (71, 72). Two SP1 binding sites closest to the transcripational start site function together to facilitate transcriptional activation by estrogen (63). In mice, an analogous region also containing a TATA box is located just upstream of the noncoding exon but this has not been assessed for promoter activity. Several alternatively spliced Kiss1 transcripts have been identified in mice that contain sequences from the first exon of the Golt1a gene (NCBI transcript accession numbers: AY707856 AY707857 AY707859). Thus Kiss1 transcripts in rodents may be generated from both a Kiss1 promoter (P1) and the Golt1a promoter (P2). The physiological significance of this is not known but may allow an additional level of gene regulation in mice.

Kisspeptins were originally isolated from human placenta (55, 78) and are derived from a larger precursor protein (FIGURE 2). The longest kisspeptin is 54 amino acids in length (Kp54), but shorter kisspeptins (Kp13, Kp14) have also been isolated corresponding to the carboxy terminus of Kp54. These shorter kisspeptins may represent degradation products, but they still retain full biological activity, as does a synthetic peptide of only 10 amino acids (Kp10). Kp14 is highly conserved between species, with the final 10 amino acids showing very little variation, particularly in mammals (Table 1). A series of alanine substitutions in Kp10 have shown that amino acids 6 (F) and 10 (F or Y, depending on the species) are the most critical for receptor binding (18, 36, 79). NMR structural modeling indicates that the COOH-terminal 7 amino acids form a helicoid structure that is disrupted by these alanine substitutions (36, 79).

The distribution of kisspeptin neurons in the hypothalamus varies between species (16). In the rodent, in situ hybridization and immunohistochemistry have been used to map kisspeptin neurons to two discrete regions of the hypothalamus, the arcuate (ARC) nucleus and in a periventricular continuum of cells within the rostral part of the third ventricle, including the anteroventral periventricular nucleus (AVPV) (12, 13, 15, 31) (FIGURE 1). Humans (95), rhesus monkeys (102), and sheep (26, 110) have proportionally more kisspeptin neurons in the ARC.
than in the AVPV region. Kisspeptin expression increases in the ARC and AVPV regions during pubertal development in rodents (15, 74, 117) and monkeys (102), consistent with kisspeptin signaling initiating puberty. Kisspeptin fibers have been found in the preoptic area (POA) of adult female rats (54) and mice (15) in close association with GnRH neuron cell bodies. These associations are probably from AVPV kisspeptin neurons and are proposed to modulate the preovulatory GnRH/LH surge in females (34). Kisspeptin immunoreactive fibers originating from cell bodies in the ARC make close apposition to GnRH axons in the median eminence of the monkey (89) and are proposed to modulate the pulsatile release of GnRH (FIGURE 1).

In some species, anatomical differences in the hypothalamus are found between the sexes with some nuclei showing a neuronal density difference (105). In rodents, the AVPV region is sexually dimorphic (106, 107) with a greater number of kisspeptin neurons in females compared with males (15, 50). This sexual dimorphism is caused by neonatal exposure to testosterone, which is aromatized into estrogen and causes defeminization of the AVPV region. Neonatal female rats treated with androgens (50) or estradiol (44) develop very few kisspeptin neurons in the AVPV, whereas castration of neonatal male rats increases the number of kisspeptin neurons in the AVPV (44). The role that estrogens play in this process has been examined by using transgenic mice lacking alpha-feto-protein (Afp\(^{−/−}\)), which protects the fetal brain from the defeminizing action of circulating estrogens. As expected, female Afp\(^{−/−}\) mice developed male-like numbers of kisspeptin neurons in the AVPV (29). Gpr54\(^{−/−}\) male mice have a feminized AVPV region with a greater number of kisspeptin neurons than wild-type males and similar to that of females (51). These data suggest that kisspeptin signaling during the neonatal period is required for testosterone production in males to defeminize the AVPV. The AVPV region also contains a sexually

**FIGURE 1. The mammalian hypothalamic pituitary gonadal axis**

At puberty, pulsatile secretion of gonadotrophic releasing hormone (GnRH) stimulates the anterior pituitary to release the gonadotrophic hormones, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These act on the gonads to promote gamete formation and the production of gonadal steroid hormones, which form feedback loops to regulate GnRH, LH, and FSH release. Kisspeptin (Kiss1) neurons act as a principal relay for steroid feedback on GnRH secretion. In females, high levels of estrogens and progesterone stimulate kisspeptin neurons of the AVPV to induce the preovulatory surge of GnRH/LH, whereas they inhibit KISS1 expression in the arcuate nucleus (ARC). In the male, GnRH and gonadotrophic hormone release are negatively regulated by circulating testosterone, partly through the activity of kisspeptin neurons of the ARC. POA, preoptic area; AVPV, anteroventral periventricular nucleus; ME, median eminence.
dimorphic population of tyrosine hydroxylase (TH) neurons (105–107), but there is little overlap between these and the kisspeptin neurons (50). In contrast, the ARC does not display sexually dimorphic differences in kisspeptin neuron density or distribution in rodents (44, 50). In sheep, however, the ARC is sexually dimorphic with half the number of kisspeptin neurons in rams compared with ewes (10). These sexually dimorphic kisspeptin neurons co-express dynorphin (DYN) and neurokinin B (NKB). Prenatal testosterone treatment of females, however, reduces DYN and NKB expression but not kisspeptin expression, suggesting different critical periods for sexual differentiation of these genes (10).

Kisspeptins and the Reproductive Axis

The critical role that kisspeptins play in regulating the reproductive axis is illustrated by the consequences of mutations in the kisspeptin signaling pathway in mice and humans. Mice with disruption of the kisspeptin receptor gene (Gpr54/Kiss1r) (24, 27, 51, 60, 100) or Kiss1 (20, 27) do not undergo pubertal development, and both sexes are infertile, with poor gonadal growth and impaired gametogenesis. Male mice have delayed spermatogenesis and produce low numbers of spermatozoa. Female mice do not show a normal estrus cycle, fail to ovulate, and do not have corpora lutea in their ovaries. This phenotype is caused by low levels of gonadotrophic hormones (LH, FSH) and gonadal sex steroids in the bloodstream (20, 24, 51, 60, 100). Similarly, loss-of-function mutations in GPR54/KISS1R in humans cause hypogonadotrophic hypogonadism (21, 59, 100, 101), whereas gain-of-function mutations cause precocious puberty (118). Moreover, a missense mutation in the kisspeptin precursor protein has been found in a Brazilian boy with central precocious puberty (104). The mutation reduces serum protease degradation of the protein and may therefore be associated with increased kisspeptin signaling.

These reproductive defects can be directly attributed to the action of kisspeptins in the hypothalamus. Central or peripheral injection of kisspeptin stimulates gonadotrophin secretion in most species, including humans (22, 23), monkeys (87, 99), sheep (4, 69), pigs (62), goats (38), rats (74–76, 119, 120), mice (31, 69), and goldfish (64). This gonadotrophin secretion is mediated by kisspeptin-stimulated GnRH release from hypothalamic neurons. Consistent with this, the majority of GnRH neurons express the kisspeptin receptor (GPR54/KISS1R), as shown by in situ hybridization or tagged expression of a LacZ reporter gene (37, 40, 46, 69, 81). Kisspeptin-mediated GnRH release has been demonstrated directly in ewes (69) and female rhesus monkeys (52) and by inhibition of kisspeptin responses in rodents by administration of GnRH antagonists (46). In addition, mice with a disrupted Gpr54/Kiss1r gene cannot secrete GnRH from hypothalamic fragments after kisspeptin stimulation (19).

Sex steroids provide feedback loops that allow the gonads to communicate with the hypothalamus to regulate GnRH release (FIGURE 1). Sex steroids achieve this indirectly, however, since GnRH neurons do not express androgen or estrogen (ERα receptors) (41, 45). It is now thought that kisspeptin neurons mediate the actions of sex steroids on GnRH neurons. The majority of kisspeptin neurons express estrogen receptor alpha (ERα of ∼90%) (26, 111, 112), the androgen receptor (∼65%) (112), and the progesterone receptor (∼86%) (110), consistent with their role as mediators of sex steroid feedback on the reproductive axis. In rodents, sex steroids

FIGURE 2. Generation of kisspeptins from the Kiss1 gene

The kisspeptin coding region (pale blue) is located within two exons of the Kiss1 gene. At least one upstream noncoding exon (white) has been identified and the promoter (P1) in humans had been mapped immediately upstream of this exon. The exact location of the mouse Kiss1 promoter remains to be determined since Kiss1 transcripts fused to Golt1a sequences have been found, suggesting some expression from a second promoter (P2) in this species. The primary translation product is a 138- to 145-amino acid protein (Kp145), depending on the species, which contains a secretory signal sequence (dark green). Proteolytic cleavage generates a 54-amino acid amidated peptide (Kp54 also known as metastin). In the placenta, shorter kisspeptins have been isolated (Kp14 and Kp13), which may be degradation products from Kp54. The shorter kisspeptins all contain the same 10 amino acids (yellow). A synthetic peptide containing only these 10 amino acids (Kp10) retains biological activity in vivo.
Kiss1

Kisspeptin action on GnRH neurons

Kisspeptin signaling through GPR54/KISS1R couples to $G_{q/11}$ to activate phospholipase C and increases inositol triphosphate (IP3) and diacylglycerol (DAG) levels in the cell (55, 73, 116) (FIGURE 3). The subsequent increase in intracellular Ca$^{2+}$ and DAG activates protein kinase C and initiates a kinase phosphorylation cascade resulting in phosphorylation of ERK1/2. DAG also stimulates GnRH depolarization by activation of a nonselective cation channel (TRPC) and inhibition of an inwardly rectifying potassium channel ($K_{r}$) (66, 84). Thus kisspeptins act directly on GnRH neurons to induce a sustained depolarization event and increase the rate of action potential firing (37, 88). The number of GnRH neurons that can respond to kisspeptins increases during puberty even though the expression levels only change slightly, suggesting posttranslational maturation of GPR54/KISS1R function with puberty (37). In adult mice, $\gamma$-amino-butyric acid (GABA) has a predominantly hyperpolarizing effect on GnRH neurons to inhibit GnRH release (14). Kisspeptins have been reported to suppress the inhibitory effects of GABA$_{\text{R}}$ receptor signaling in GnRH neurons (123). Thus, under conditions of estrogen positive feedback when kisspeptin levels increase in the AVP, kisspeptins will antagonize any inhibitory action of GABA to stimulate GnRH release and LH surge/ovulation.

After initial stimulation, continuous administration of kisspeptin suppresses the reproductive axis. Continuous delivery of human Kp10 inhibited further LH release in agonadal monkeys within 24 h (90, 99). Similarly, sustained kisspeptin activity in women failed to elicit LH or FSH release by day 14 (49). In contrast, pulsatile delivery of kisspeptin in agonadal juvenile monkeys did not suppress gonadotropic hormone release (87). The suppressive effects are probably caused by desensitization of GPR54/KISS1R rather than the GnRH receptor since all individuals remained responsive to GnRH.

Desensitization of GPR54/KISS1R signaling is mediated by G-protein-coupled receptor serine/threonine kinase 2 (GRK2) and $\beta$-arrestin in a cell culture model (80). GPR54/KISS1R also displays constitutive basal activity at ~5% of the maximum activity after kisspeptin stimulation (80). This basal activity may explain why the phenotype of Kiss1$^{-/-}$ mutant mice is less severe than that of Gpr54$^{-/-}$ mutant mice (9, 60).

Kisspeptins may also have indirect effects on GnRH neurons via synaptic input from other neurons in the hypothalamus that express GPR54/KISS1R rather than the GnRH receptor. Part of the GnRH response to kisspeptins is mediated by trans-synaptic input from other neurons. Under these conditions, the GnRH response to kisspeptins is mediated by trans-synaptic input from GABAergic and glutamatergic neurons, which was differentially involved in the regulation of kisspeptin expression to decrease expression in the AVPV region (50, 111) and increase expression in the ARC (111). These changes are reversed by either testosterone or estradiol replacement (111, 112). Studies in other species have corroborated the effects of sex steroids on kisspeptin expression (46, 74, 95, 103, 110). In the sheep, the ARC kisspeptin neurons mediate both the negative feedback action of progesterone on GnRH release during the luteal phase (11) and the positive feedback action of estradiol expression at the time of ovulation (25, 113). The mechanism by which sex steroids differentially regulate kisspeptin expression in the AVPV and ARC regions had not been resolved, but estradiol mediates its feedback effects through both estrogen response element (ERE)-dependent and ERE-independent signaling in rodents. This was shown by examining the effects of estradiol on Kiss1 expression in mutant mice with Erα molecules that cannot bind to ERE sequences (32). Stimulation of Kiss1 expression by estradiol in the AVPV required an ERE-dependent pathway. In contrast, inhibition of Kiss1 expression by estradiol in the ARC was ERE-independent (32). In addition, insulin-like growth factor-1 (IGF-1) increases Kiss1 expression in the AVPV but not the ARC of prepubertal female rats in the presence of estradiol (42, 43). These expression changes are consistent with kisspeptin neurons in the ARC regulating the negative feedback effect of sex steroids on GnRH release and those in the AVPV/PeN regions responsible for the preovulatory GnRH/LH surge (39) (see FIGURE 4).

<table>
<thead>
<tr>
<th>Species</th>
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<td>Zebrafish</td>
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Kp14 is highly conserved between many species. The leucine$^2$ (L) to valine (V) or methionine (M) are conserved amino-acid substitutions (58). Differences in the human sequence are shown in italicized bold.
enhanced by estradiol (84, 85). Moreover, kisspeptin increased inhibitory GABAergic input to GnRH neurons during the estradiol-mediated negative feedback part of the estrous cycle (84, 85).

**Physiological Regulation of Kisspeptin Signaling**

Mammalian ovulation requires an LH surge brought about by the positive feedback action of estradiol on GnRH release. Kisspeptin expression increases in the AVPV region just before ovulation and during a steroid-induced LH surge in ovariectomized rats (114). At the time of ovulation, kisspeptin neurons in the AVPV are activated as indicated by induction of c-fos (13). Inhibition of kisspeptin action in the POA by local injection of a monoclonal antibody abolishes the proestrous LH surge and inhibits estrous cyclicity in rats (1, 54). In the absence of either GPR54/KISS1R or kisspeptin expression in transgenic female mice, the sex steroid-induced GnRH/LH surge does not occur compared with wild-type siblings (13). In rodents, tissue ablation studies have shown that the LH surge is also gated by a circadian oscillator in the suprachiasmatic nucleus (SCN) to ensure that the surge occurs near the onset of darkness. Kisspeptin expression and c-fos neuronal activation in the AVPV region are also subject to circadian regulation. This was demonstrated in ovariectomized mice that were maintained in constant darkness and still showed an estradiol-induced LH surge (94). Thus kisspeptin signaling, regulated by both estradiol and circadian signals, is essential for the preovulatory GnRH/LH surge.

Kisspeptin signaling may also play a role in modulating the pulsatile release of GnRH. The mechanism by which this occurs is not fully understood, but recent information about the gene expression profile of kisspeptin neurons has provided important clues. In sheep and mice, the majority of kisspeptin neurons in the ARC co-express dynorphin A (DYN) and neurokinin B (NKB) (30, 77) (FIGURE 4). Of mouse ARC kisspeptin neurons, 96% also express the neurokinin B receptor gene Nk3r, but only 20% of kisspeptin neurons express the dynorphin receptor gene Kor. This may be an underestimate, however, since others have shown by immunohistochemistry that practically all NKB-positive neurons in the rat ARC co-express DYN (3). In contrast, kisspeptin neurons in the AVPV regions of the female mouse hypothalamus show limited expression of Dyn (33%) and NKB (10%). Co-expression of DYN and NKB in kisspeptin neurons may be physiologically relevant since both of these neuropeptides can inhibit LH secretion in rats (53, 77, 97, 98), and there is an intimate association between ARC kisspeptin/NKB neurons and GnRH fibers at the median eminence (56, 89). Kisspeptin/NKB/DYN neurons also form connections with each other (57), and this has led to the suggestion that signaling between these neuropeptides may coordinate the release of kisspeptin at GnRH nerve terminals to generate pulsatile release of GnRH (57, 77) (FIGURE 4). In this scheme, NKB is proposed to synchronize and stimulate DYN production in all kisspeptin neurons. DYN would then reduce NKB secretion by negative feedback, which would consequently reduce DYN levels to generate regular pulses of NKB and DYN. Pulsatile kisspeptin release would be driven by these pulsatile changes in NKB and DYN, which signal through different G-proteins and could thereby act to differentially regulate kisspeptin release. Interestingly, Kiss1, Nk3, and Dyn all show similar negative regulation in expression by estradiol (77, 91, 111). In support of this hypothesis is the observation that infusion of a selective kisspeptin antagonist into the ARC nucleus can reduce LH pulse frequency but not pulse amplitude in female rats (65). Whatever function NKB signaling has in coordinating pulsatile kisspeptin and GnRH release, it seems to be less physiologically important in mice since Nk3r knockout mice are not reported to show any reproductive defects (108). This is in contrast to loss-of-function mutations in NKB or Nk3R in humans, which are associated with reproductive failure (28, 35, 121).

The way in which NKB acts on GnRH neurons is not clear. In rats, GnRH neurons express the c-fos and its induction is proposed to synchronize and stimulate DYN production (211). Inhibition of NKB/Nk3r signaling in transgenic female mice, the sex steroid-induced GnRH/LH surge does not occur compared with wild-type siblings (108). This is in contrast to loss-of-function mutations in NKB or Nk3R in humans, which are associated with reproductive failure (28, 35, 121).
neurokinin B receptor (NK3R) on axons in the median eminence but not at the GnRH cell bodies (56). In contrast, NK3R expression has not been found in GnRH neurons of ewes. Although GnRH fibers in the median eminence of rats express the NK3R, the physiological action of NKB on GnRH neurons is not clear. Some groups have reported inhibition of LH secretion after central administration of the neurokinin B agonist senktide in the rat (77, 97), whereas others have observed little effect in mice (17). In ewes, senktide stimulates LH release during the follicular phase but not the luteal phase of the oestrus cycle (68). These disparities may reflect the difference in NK3R expression between species, with senktide having a dual action on both GnRH and kisspeptin neurons in rodents but only an indirect action via kisspeptin neurons in sheep. NK3R and GPR54/KISS1R both signal via Gq/11, so they may be expected to show synergistic activities. Indeed, central administration of NKB and Kp10 produced greater LH secretion in male mice than with Kp10 administration alone (17).

Kisspeptin signaling is also involved in regulating the reproductive cycle of species that show seasonal breeding. Several species have seasonal breeding patterns that coordinate time of birth with optimal environmental conditions. A reduction in kisspeptin expression during the nonbreeding (anoestrous) season has been found in sheep (110) and hamsters (33, 92). Administration of

**FIGURE 4. Interaction of kisspeptin neurons with GnRH neurons in rodents**

Kisspeptin neurons are found mainly in the anteroventral periventricular (AVPV) and arcuate (ARC) regions of the rodent hypothalamus. Gonadotrophin releasing hormone (GnRH) neurons are located in the preoptic area (POA) and send fibers to the median eminence (ME). Kisspeptin neurons in the AVPV are sexually dimorphic with greater numbers in females and are thought to regulate the preovulatory GnRH/LH surge. Kisspeptin neurons in the ARC co-express neurokinin B (NKB) and dynorphin (DYN). The kisspeptin receptor (GPR54/KISS1R) and the neurokinin B receptor (NK3R) are both expressed at GnRH nerve terminals. The kisspeptin neurons from the ARC form close appositions with GnRH terminals in the inner zone of the median eminence and also project to each other. NK3R and GPR54/KISS1R are coupled to Gq/11, whereas the DYN receptor (KOR) is coupled to a Gi/o. Inset: dynorphin and neurokinin B signaling between kisspeptin neurons may be involved in synchronizing kisspeptin release to generate GnRH pulsatility. +, Stimulatory activities; −, inhibitory activities. NKB and DYN receptors signal through different G-proteins, which may allow differential regulation of kisspeptin release.
Kisspeptins during the nonbreeding season can stimulate the reproductive axis and induce testicular growth in hamsters (92) and ovulation in ewes (4). Signals controlling these seasonal changes in kisspeptin expression may include photoperiod acting via melatonin (8, 92) and food restriction (82).

Kisspeptins also play a role in integrating signals about metabolic status to the reproductive axis. Low body weight associated with undernutrition can suppress the reproductive axis, and food restriction has been shown to decrease kisspeptin expression in adult rodents (5, 67). Undernutrition during fetal development may also affect kisspeptin expression and influence reproductive function in adulthood. Prenatal undernutrition in rats results in reduced Kiss1 expression at postnatal day 16 and delayed vaginal opening, which can be reversed by chronic central injection of kisspeptin (47). Mice that are deficient in leptin (Ob/Ob) have reduced kisspeptin expression compared with wild-type, which is reversed by leptin infusion (67).

Similarly, central administration of leptin normalizes the low kisspeptin levels found in an experimentally induced diabetic rat model (6, 7). Around 40% of kisspeptin neurons in the ARC co-express the leptin receptor (109), suggesting that kisspeptin neurons monitor peripheral fat reserves via leptin to modulate the reproductive axis under conditions of negative energy balance. Modulation of kisspeptin expression by leptin may be mediated by the mammalian target of rapamycin (mTOR) protein since mTOR activation stimulates the reproductive axis and inactivation reduces kisspeptin expression in the ARC (93). Melanin-concentrating hormone (MCH) may also provide a link between energy status and reproduction since this hormone is upregulated during fasting and can inhibit the action of kisspeptin on some GnRH neurons (122).

Other factors that influence the reproductive axis may do so via kisspeptin neurons. Melanocortins, which stimulate LH release in several mammalian species, have been shown to increase Kiss1 expression in the dorsolateral POA after infusion of an agonist into the lateral ventricle of luteal stage ewes (2). Short-term exposure to alcohol has also been shown to reduce Kiss1 expression in the AVPV and ARC nuclei (115). These affects of alcohol may be mediated by reduced insulin-like growth factor 1 (IGF-1) signaling since alcohol reduces IGF-1 levels in the bloodstream (115) and IGF-1 can stimulate Kiss1 expression in the AVPV region of prepubertal female rats (42, 43).

**Kisspeptin Agonists and Antagonists**

Kisspeptin analogs with agonistic or antagonistic activities could be useful for the treatment of clinical disorders such as infertility (48), premature or delayed puberty, and prostatic or metastatic cancers. Analogs need to be tested in vivo since receptor binding affinities in cell culture do not always predict biological potency in a living animal. For example, all kisspeptin family members bind to GPR54/KISS1R with similar affinities (55), but Kp54 is more potent than Kp10 in rodents after peripheral injection (70, 83, 119), probably due to differences in bioavailability. Thus development of Kp10 analogs with greater in vivo stability than Kp10 may help in the development of therapeutic products. A Kp10 analog in which the amino-terminal tyrosine (Y) is replaced with an enantionic tyrosine (dYNWNSFGLRF-NH₂, [dY]1Kp10) has similar receptor binding and signaling activity to Kp10 but shows more potent effects in vivo (18). Peripheral administration of [dY]1Kp10 increased plasma LH and testosterone levels in male mice more potently than Kp10 (18), possibly due to reduced degradation of the analog.

The development of kisspeptin antagonists should also allow an assessment of the action of kisspeptin on specific parts of the hypothalamus or at defined times during the estrous cycle or pregnancy. These temporospatial studies are not possible in the Gpr54/Kiss1r or Kiss1 knockout mice, which have congenital absence of kisspeptin signaling in all tissues. Roseweir and colleagues (96) generated a Kp10 antagonist (dANWNGFGdWRF, peptide 234) that has two D-amino acid substitutions and a Ser to Gly change at position 5. In a comprehensive analysis, peptide 234 was shown to potently inhibit Kp10 signaling in CHO cells expressing GPR54/KISS1R and decrease Kp10-stimulated GnRH neuron firing in brain slice preparations. In vivo, central delivery of peptide 234 inhibited pulsatile GnRH release in pubertal female rhesus monkeys and pulsatile LH secretion in ovarioctomized ewes. In rodents, peptide 234 reduced the postcastration rise in LH and attenuated Kp10-stimulated LH secretion (96). Continuous central infusion of peptide 234 delayed puberty in female rats and prevented the preovulatory LH surge in sexual mature rats (86). Peptide 234 is probably acting as a competitive inhibitor with better bioavailability than Kp10. Inhibition effects are typically observed when peptide 234 is used at a ×10–1,000 molar excess over Kp10.

For maximum utility as a therapeutic agent, kisspeptin analogs should be capable of crossing the blood-brain barrier after peripheral administration. Whether peptide 234 can cross the blood-brain barrier has not been completely established. Systemic injection of peptide 234 fused with penetratin, a cationic cell-penetrating peptide, can inhibit LH and FSH release in response to central injection of Kp10 (86). This does not prove that the
fusion protein can cross the blood-brain barrier, however, since inhibition may be at the level of the median eminence where the centrally injected Kp10 may still act, and it was not reported whether the same effects were found with peptide 234 lacking penetratin.

Summary and Unanswered Questions

Kisspeptin signaling through the G-protein-coupled receptor, GPR54/KISS1R, is crucial for initiation of puberty and maintenance of mammalian fertility. Kisspeptin neurons act as central integrators of external and physiological signals within the hypothalamus. They are potent stimulators of GnRH release and mediate sex steroid feedback on the reproductive axis. Kisspeptin neurons in the arcuate region of the hypothalamus regulate the tonic pulsatile release of GnRH, whereas those in the AVPV generate the preovulatory LH surge in females. There are still unresolved questions, however, such as which molecules act upstream of Kiss1 to regulate expression and what the mechanism is by which the two populations of kisspeptin neurons (AVPV and ARC) are differentially regulated by sex steroids. In addition, does NKB/DYN signaling regulate the activity of ARC kisspeptin neurons and what other neuropeptides may be involved? Why do NK3R mutations cause infertility in humans but not in mice? Also, is it true that the AVPV neurons are solely responsible for the GnRH/LH surge in females, whereas the ARC neurons act at GnRH nerve terminals to regulate tonic pulsatile GnRH/LH release? Finally, what role does GPR54/KISS1R oligomerization or interaction with other membrane receptors have in modulating the activity of kisspeptins on GnRH neurons? The answers to these questions should provide us with important knowledge about the central mechanisms controlling the mammalian reproductive axis.

No conflicts of interest, financial or otherwise, are declared by the author(s).

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