Journal of Reproduction and Development, Vol. 62, No 6, 2016

—Review—

The roles of kisspeptin revisited: inside and outside the hypothalamus

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Abstract. Kisspeptin, encoded by *KISS1*/*Kiss1* gene, is now considered a master regulator of reproductive functions in mammals owing to its involvement in the direct activation of gonadotropin-releasing hormone (GnRH) neurons after binding to its cognate receptor, GPR54. Ever since the discovery of kisspeptin, intensive studies on hypothalamic expression of *KISS1*/*Kiss1* and on physiological roles of hypothalamic kisspeptin neurons have provided clues as to how the brain controls sexual maturation at the onset of puberty and subsequent reproductive performance in mammals. Additionally, emerging evidence indicates the potential involvement of extra-hypothalamic kisspeptin in reproductive functions. Here, we summarize data regarding kisspeptin inside and outside the hypothalamus and revisit the physiological roles of central and peripheral kisspeptins in the reproductive functions of mammals.

Key words: Follicle-stimulating hormone, Gonadotropin-releasing hormone, Kisspeptin, Luteinizing hormone, Puberty

(**J. Reprod. Dev. 62:** 537–545, 2016)

Introduction

In the late 1940s, Harris [1] predicted the presence of hypothalamic releasing hormones, which are conveyed from the median eminence to the pituitary gland through the hypophyseal portal circulation to control the synthesis and secretion of pituitary hormones such as gonadotropins. This opened the door to the discovery of the hypothalamic releasing hormones. After intensive studies on the predicted hormones, two independent groups led by Schally [2] and Guillemin [3], the 1977 Nobel laureates, isolated luteinizing hormone-releasing hormone (LHRH), a ten-amino-acid neuropeptide, from the hypothalamus of pigs and sheep. This decapeptide stimulates both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in several mammals [4] and, therefore, has been termed as the 'gonadotropin-releasing hormone' (GnRH).

The importance of GnRH secretion in mammalian reproduction was demonstrated by the pioneer experiments of Knobil and his colleagues in the late 1970s and early 1980s. They had established a GnRH replacement regimen to deliver GnRH in a pulsatile manner at a rate equivalent to the physiological frequency of LH pulses [5, 6], which was observed in ovariectomized rhesus monkeys using radioimmunoassays [7]. This regimen successfully recovered both FSH and LH levels in female monkeys bearing hypothalamic lesions that abolished gonadotropin secretion [5]. In addition, the regimen successfully induced regular menstrual cycles and the withdrawal of GnRH reverted prepubertal female monkeys to an immature state [8]. The necessity of the pulsatile nature of tonic GnRH secretion can be understood in light of the regulation of GnRH receptors by GnRH itself in gonadotrophs of the anterior pituitary, because the continuous infusion of GnRH secretion abolished gonadotropin secretion in female monkeys [5]. Tonic FSH and LH secretion is found to be pulsatile in males and in most phases of estrous or menstrual cycles in females [9–12]. A positive relationship between GnRH and LH has been clearly demonstrated in sheep where each GnRH pulse in the hypophyseal portal blood corresponds to each LH pulse in peripheral circulation [13]. The tonic gonadotropin secretion is fine-tuned by negative feedback action of circulating estrogen derived from the ovarian follicles [14]. The mechanism of the feedback action, however, is largely unknown.

Besides the pulse mode of GnRH/gonadotropin secretion, the surge mode of GnRH release is characterized by a large amount of GnRH/gonadotropin secretion in females. The GnRH surge-induced LH surge is required to induce ovulation, a critical event in female reproduction [15–17]. As ovarian follicles grow larger and become mature, high levels of circulating estrogen exert their positive impact on GnRH neurons to induce a GnRH surge and hence the LH surge in female mammals. The GnRH surge in the hypophyseal portal circulation has clearly been demonstrated to correspond to the LH surge in peripheral circulation in sheep [17–19]. Administration of high doses of estrogen reproduces GnRH/LH surges in gonadectomized females in diverse mammalian species [16, 17, 20]. On the other hand, the response to high doses of estrogen in castrated males varies from species to species. Administration of high doses of estrogen induces surge-like LH secretion in male primates [21–24] and goats [25, 26], but not in sheep [27] and rodents [28–30]. Thus, the brain mechanism generating GnRH/LH surges (also known as the mechanism underlying the positive feedback action of estrogen) is likely conserved in the males of primates and goats, whereas it

Received: May 24, 2016
Accepted: July 1, 2016
Published online in J-STAGE: July 29, 2016

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appears sexually differentiated in sheep and rodents.

The molecular and cellular mechanism underlying the negative and positive feedback actions of estrogen has been a big concern in reproductive physiology for years. This is primarily because GnRH neurons do not express estrogen receptor α (ERα) [31], which is considered to mediate both types of estrogen feedback actions. The most plausible explanation was that other ERα-expressing neurons receive and transmit estrogen signals to the GnRH neurons and hence properly control GnRH neuronal activities during the estrous cycle in females [14, 32, 33]. In recent years, the two populations of hypothalamic kisspeptin neurons are found to be the targets of estrogen negative/positive feedback actions in order to control two modes (pulses and surges) of GnRH/gonadotropin secretion in mammals. In addition, emerging evidence indicates the potential physiological roles of extra-hypothalamic kisspeptin, produced in gonads, uteri, and placenta, on reproductive performance in mammals. The present review thus focuses on the roles of kisspeptin inside and outside the hypothalamus. A brief review of the discovery of kisspeptin is followed by a discussion of the physiological roles of kisspeptin in controlling gonadal functions in mammals.

Discovery of Kisspeptin and Its Cognate Receptors

KISS1 gene [34] and its translation product [35] was first identified as a gene and protein responsible for metastasis suppression in humans. *KISS1* was isolated from human nonmetastatic melanoma cells in 1996 [34]. The processed 54-amino-acid peptide *KISS1* translation product was identified in human placenta as an endogenous ligand of GPR54, a galanin receptor-like orphan G-protein coupled receptor, in 2001 [35]. The peptide exhibited the ability to suppress tumor metastasis and was therefore designated as metastin [35]. A high level of *KISS1* expression was found in healthy and tumorous human tissues [35]. Concurrently, *KISS1* translation products were found in human placenta as endogenous ligands of GPR54 and designated as kisspeptins [36]. To date, the term kisspeptin has been preferably used in the field of reproductive biology after discussion at the First World Conference of Kisspeptin Signaling in the Brain, in Cordoba in 2008.

The amino acid sequence of processed kisspeptin deduced from cloned cDNA consists of 52 to 54 amino acids and is well conserved in most mammals examined to date [35–41]. In particular, the C-terminal-amidated 10-amino acid sequence, which is considered to bind to GPR54 [35], is identical among mammalian species, except for tyrosine at the C-terminal being changed to phenylalanine in primates. Primate kisspeptin contains the RF-amide motif at the C-terminal, and thus kisspeptin is classified as a member of the RF-amide peptide family [42]. Ours and other previous studies showed that human kisspeptin activated intracellular signaling by exhibiting potent binding affinity to GPR74 and GPR147, that are known receptors for neuropeptide FF and RF-amide-related peptide [43, 44], which are other members of the RF-amide peptide family. The physiological significance of kisspeptin-GPR74/147 signaling in reproductive biology, if any, remains to be determined. In light of this promiscuous relationship between peptides and receptors, the term *GPR54*/*Gpr54* is used in this review instead of *KISS1R*/*Kiss1r*, the official gene symbol of the kisspeptin receptor.

Kisspeptin as a Gatekeeper of Puberty Onset in Mammals

The first evidence for the physiological significance of kisspeptin-GPR54 signaling in the onset of puberty dates back to two independent findings of loss-of-function mutations of *GPR54* in humans with hypogonadotropic hypogonadism, a pubertal failure with impaired secretion of gonadotropins [45, 46]. One of these research groups generated *Gpr54* knockout mice and showed the *Gpr54* knockout mice successfully reproduced the phenotype of human *GPR54* mutations [46]. These findings strongly suggest that GPR54 and its endogenous ligand kisspeptin play a key role as gatekeepers of sexual maturation at the onset of puberty. To date, the phenotype of *GPR54* mutations in humans was recapitulated in loss-of-function mutations of *KISS1* in humans [47] and in several animal models carrying targeted mutations in *Kiss1* or *Gpr54* loci [48–54].

Further analysis of the first *Gpr54* knockout mouse line, which was generated by *LacZ* insertion in *Gpr54* locus, revealed β-galactosidase activity representing GPR54 expression in normally migrated GnRH neurons [49]. Kisspeptin, therefore, is a key molecule that directly controls GnRH neurons, as opposed to contributing to the migration of GnRH neurons from the nasal placode in mammals. Indeed, kisspeptin or its C-terminal amidated decapeptide (also known as Kp-10) profoundly stimulates gonadotropin secretion via GnRH secretion [55–57]. These findings suggest that kisspeptin is a potent stimulator of GnRH secretion via GPR54 expressed in GnRH neurons. Recently, we generated *Kiss1* knockout rats to evaluate the hormonal profiles in *Kiss1* knockout animal models in more detail [54]. The *Kiss1* knockout rats exhibited a lack of both pulse and surge modes of gonadotropin (both LH and FSH) secretion and failure of puberty onset, indicating that kisspeptin plays an indispensable role in generating tonic and cyclic GnRH secretion to regulate puberty onset and normal reproductive performance. It should be noted that the *Kiss1* knockout male rats exhibit no male sexual behaviors, but showed female-like lordosis reflex, suggesting that kisspeptin is also indispensable for the defeminization and masculinization of the brain mechanism controlling sexual behaviors in male rats [58].

Hypothalamic Kisspeptin Neurons Control GnRH Secretion

Two populations of hypothalamic kisspeptin neurons

KISS1/Kiss1 expression and its localization in the brain were extensively examined in several mammalian species. Localization of hypothalamic kisspeptin neurons is largely similar in all mammalian species examined [24, 26, 39, 40, 59–63]. Hypothalamic kisspeptin neurons are mainly localized in two regions: the anterior region of the hypothalamus called the anteroventral periventricular nucleus (AVPV) in rodents, or the preoptic area (POA) in other species, and the posterior region of the hypothalamus called the arcuate nucleus (ARC). As shown below, kisspeptin neurons localized in the AVPV of rodents are possibly equivalent to those in the POA of goats and monkeys. In addition to the two major populations, there are a few additional small populations of kisspeptin neurons in the hypothalamus, such as ventromedial hypothalamus and paraventricular nucleus [64, 65]. Xu *et al*. [64] suggested a potential role of those

kisspeptin neurons in reproductive behavior. This supposition is consistent with our recent study showing that *Kiss1* knockout rats exhibit abnormal sexual behavior [58].

The two major populations of kisspeptin neurons localized in the POA/AVPV and ARC are considered to have separate roles in female reproduction, because earlier studies in rodents demonstrated a different pattern of *Kiss1* expression in these two hypothalamic regions. Briefly, AVPV *Kiss1* expression is highest in the afternoon of proestrus and is positively regulated by estrogen, whereas ARC *Kiss1* expression is negatively regulated by estrogen treatment in rodents [60, 62, 66, 67]. It is, therefore, likely that the AVPV kisspeptin neurons are a target of estrogen positive feedback action and hence generate the GnRH surge and that the ARC kisspeptin neurons are a target of estrogen negative feedback action and are involved in GnRH pulse generation. The bidirectional regulation of *Kiss1* expression by estrogen might be mediated by ERα, because estrogen changes AVPV or ARC *Kiss1* expression in ovariectomized ERβ knockout mice, but not in ERα knockout mice [60]. Recent advances in epigenetic research for the regulation of *Kiss1* expression [68, 69] provide a clue as to how estrogen regulates *Kiss1* expression in these two hypothalamic regions in a different manner. In the AVPV, estrogen-ERα complexes, which are highly recruited at the *Kiss1* promoter, are likely involved in the histone acetylation of the *Kiss1* promoter and the subsequent *Kiss1* expression by a chromatin loop formation between the *Kiss1* promoter and the 3′-downstream enhancer region [68]. In the ARC, histone acetylation of the *Kiss1* promoter and a chromatin loop formation between the *Kiss1* promoter and the 5′-upstream enhancer region seems to be involved in *Kiss1* expression in the absence of estrogen [68, 69]. Taken together, histone acetylation of the *Kiss1* promoter is positively correlated with *Kiss1* expression in both hypothalamic nuclei, and region-specific enhancers serve as switches for the bidirectional regulation of *Kiss1* expression (Fig. 1). Further studies are warranted to elucidate how the histone acetylation of the *Kiss1* promoter region is bidirectionally regulated by estrogen in the AVPV and ARC kisspeptin neurons.

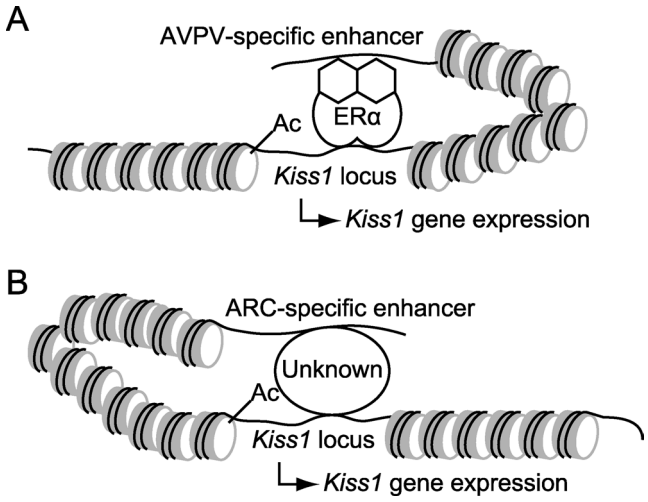

Fig. 1. Schematic illustration of the molecular and epigenetic mechanism underlying hypothalamic *Kiss1* expression. (A) In the anteroventral periventricular nucleus (AVPV), estrogen-estrogen receptor α (ERα) complex induces *Kiss1* expression via histone acetylation of the *Kiss1* promoter and chromatin loop formation between the *Kiss1* promoter region and the 3'-downstream region. (B) In the arcuate nucleus (ARC), histone acetylation of the *Kiss1* promoter and chromatin loop formation between the *Kiss1* promoter region and the 5'-upstream region via unknown transcriptional factor(s) seems to be involved in *Kiss1* expression. Ac, histone acetylation.

Functions of the POA/AVPV kisspeptin neurons

It is well accepted that the POA/AVPV kisspeptin neurons play a key role as a surge generator, mediating the positive feedback action of estrogen to trigger the preovulatory GnRH/LH surge in female mammals (Fig. 2). The AVPV has been considered to be a site of positive feedback action of estrogen in rats for years, because estrogen microimplants in this region successfully evoked, but electrical lesion of this region abolished the LH surge in rats [20, 70]. Besides previous studies, the POA/AVPV kisspeptin neurons are well accepted to be equipped with a GnRH surge-generating mechanism. The POA/AVPV kisspeptin neurons express ERα [40, 60, 62, 67] and their *KISS1*/*Kiss1* expression is induced by estrogen in a variety of mammals [24, 26, 39, 40, 60, 62, 71]. The physiological roles of kisspeptin in the induction of LH surge were demonstrated by a blockade of preovulatory or estradiol-induced LH surges with a microinjection of anti-kisspeptin antibody into the POA in rats [62, 66], or with a central injection of a kisspeptin antagonist in sheep and rats [72, 73].

In rodents, the distribution of AVPV kisspeptin neurons is sexually differentiated: Female rodents exhibit a large number of kisspeptin neurons along the ventricle of the AVPV, whereas males show only a few scattered kisspeptin neurons in this nucleus [61, 62, 74]. The sexual dimorphism of AVPV kisspeptin neurons is caused by an organizational effect of steroids secreted by perinatal testes, because neonatal castration in male rats allows estrogen-induced AVPV *Kiss1* expression in genetically male rats [29]. On the other hand, female rats with neonatal androgen/estrogen treatment display a male-like pattern of AVPV *Kiss1* expression at adulthood [29, 74]. Thus, it is plausible that estrogen converted from perinatal testicular androgen causes defeminization of the AVPV kisspeptin system in rodents.

The sexual dimorphism in AVPV kisspeptin neurons is likely responsible for the sexually differentiated mechanism underlying LH surge generation in rodents: Orchidectomized male rodents did not show a LH surge even if they received preovulatory levels of estrogen [28–30]. There are species differences in the sexual dimorphism of the LH surge generating system, because the estrogen-induced LH surge is evident in castrated male primates [22, 24] and goats [25, 26] as described earlier in this review. Recent studies [24, 26] demonstrated preovulatory levels of estrogen-induced POA *KISS1* expression and/or c-Fos expression detected in the kisspeptin neurons in males of these species. Thus, unlike rodents, the inherent LH surge generating system seems to be conserved in male monkeys and goats at adulthood. The responsiveness to estrogen in the POA kisspeptin neurons in monkeys and goats is likely to be lower in males than females: The numbers of the POA kisspeptin neurons in male monkeys and c-Fos expressing kisspeptin neurons in male goats are fewer than those in females in the presence of preovulatory levels of estrogen [24, 26]. Thus, the POA kisspeptin neurons in

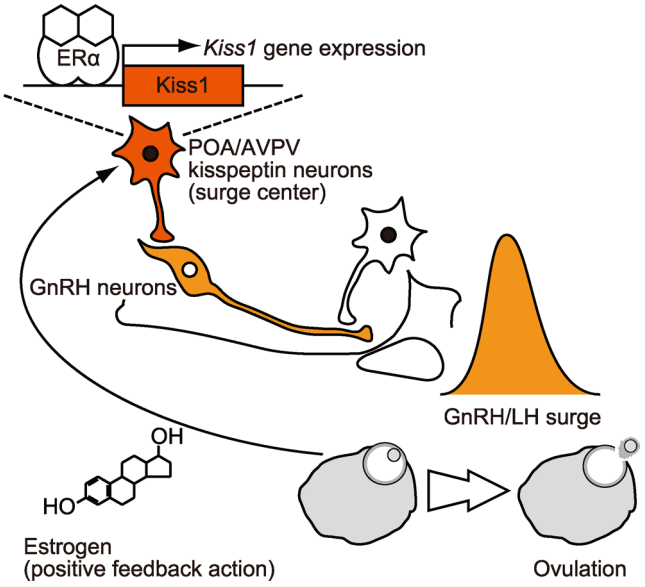

Fig. 2. Schematic illustration of the brain mechanism controlling the preovulatory gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) surge in mammals. Estrogen derived from the mature follicles exerts a positive feedback action on *Kiss1* expression in the preoptic area (POA)/AVPV. Kisspeptin appears to act on GnRH neuronal cell bodies and triggers GnRH/LH surge and subsequent ovulation in females.

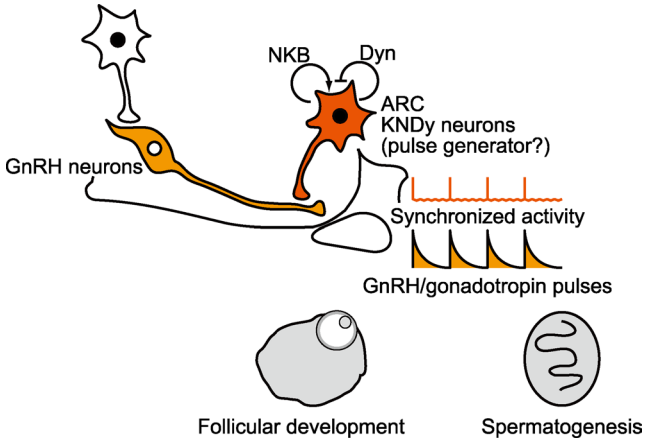

Fig. 3. Schematic illustration of the brain mechanism generating GnRH pulses in mammals. The most plausible explanation is that, in the ARC, KNDy neurons are an intrinsic source of GnRH pulses; under this condition, neurokinin B (NKB) stimulates, while dynorphin A (Dyn) inhibits, synchronized neuronal activity among KNDy neurons. The interaction of these peptides probably generates pulsatile kisspeptin secretion. Kisspeptin appears to act on the GnRH neuronal terminals and generates pulsatile secretion of GnRH and hence gonadotropin, which regulates ovarian follicular development and testicular spermatogenesis.

monkeys and goats are sensitive to testicular androgen during the developmental period as suggested in rodents and are partly defeminized by estrogen during the period. It might be dependent on species differences in the timing of androgen secretion from the fetal testes and the critical period window of brain organization and differentiation. Indeed, gestational androgen exposure induces polycystic ovary syndrome in rhesus monkeys [75], suggesting a dysfunction of the LH surge generating system. It should be noted that estrogen failed to induce the LH surge in intact male monkeys [16], probably because of the inert mechanism generating the LH surge in the presence of androgen.

The involvement of the POA kisspeptin neurons in inducing LH surge is still open to dispute in sheep. Conflicting evidence exists regarding estrogen-induced POA *KISS1* expression in ewes [63, 71]: Estrogen did not always exert a stimulatory influence on *KISS1* expression in this nucleus. Ewes exhibit higher *KISS1* expression in both the POA and ARC at the late follicular phase compared to the luteal phase [63]. Hoffman *et al*. [76] showed the activation of POA but not ARC kisspeptin neurons at the timing of LH surge in ewes. Further studies are required to evaluate the role of POA kisspeptin neurons in the induction of LH surge in ewes. The involvement of ARC kisspeptin neurons in GnRH/LH surge generation will be discussed in the next section of this review.

Functions of the ARC kisspeptin neurons

The ARC kisspeptin neurons are considered a part of the GnRH pulse generator. A method for detecting the ARC multiple unit activity (MUA) volleys has been introduced to rhesus monkeys in 1980s [77], then to rats [78], and finally goats [79]. The ARC MUA volleys correspond to LH pulses and therefore are considered to represent the activity of the GnRH pulse generator. The ARC kisspeptin neurons would be the source of the periodic MUA volleys, because MUA volleys are successfully monitored only when the recording electrodes are placed in close proximity to the kisspeptin neurons in the goat ARC [38]. The ARC kisspeptin neurons project their fibers to the median eminence, where kisspeptin fibers are closely associated with GnRH fibers in rats and goats [80, 81]. This suggests that GnRH neuronal terminals in the median eminence are one of the action sites of kisspeptin for stimulating GnRH secretion [80, 81]. In this context, Keen *et al*. [82] showed pulsatile kisspeptin secretions at the median eminence and most of the secretions were correlated with GnRH pulses in rhesus monkeys.

The morphological characteristics of the ARC kisspeptin neurons bring us a better understanding of the GnRH pulse generator. In 2007, Goodman *et al*. [83] discovered that two neuropeptides, neurokinin B and dynorphin A, are colocalized in the ARC kisspeptin neurons in sheep. Since then, the ARC kisspeptin neurons are referred to as KNDy (kisspeptin/neurokinin B/dynorphin A) neurons [84]. The colocalization of the three neuropeptides in the ARC was then confirmed in other mammals, such as mice and goats [85, 86]. Wakabayashi *et al*. [86] demonstrated that neurokinin B facilitated, and dynorphin A inhibited, the frequency of MUA volleys in goats. The effects of neurokinin B and dynorphin A on LH pulses were then confirmed in sheep [87]. These findings suggest that the KNDy neuron is an intrinsic source of the GnRH pulse generator, in which the two neuropeptides may function in an autocrine and/or paracrine manner (Fig. 3). Indeed, most KNDy neurons express tachykinin receptor 3 (NK3R), a receptor for neurokinin B, in sheep [88]

and mice [85]. Thus, it is likely that neurokinin B is involved in the generation of intermittent bursts of KNDy neurons via NK3R activation and that kisspeptin transmits the pulse generator activity toward the GnRH neurons [86, 87, 89]. On the other hand, dynorphin A has been suggested to terminate the burst activity of the GnRH pulse generator via an unknown mechanism(s), because only a small number of KNDy neurons express κ-opioid receptors, which are receptors for dynorphin A [85, 90]. The transmitter phenotypes of neurons expressing κ-opioid receptors, which are involved in GnRH pulse generation, remain an open question.

Interestingly, the ARC kisspeptin neurons might also be involved in GnRH/LH surge generation as well. In sheep, the number of ARC *KISS1*-expressing cells increased in the late follicular phase [63, 91]. Merkley *et al*. [92] suggested that an activation of the ARC kisspeptin neurons as well as their POA population determines the timing of LH surge in sheep. Similarly, the activation of kisspeptin neurons located in the ARC and AVPV of rats at the proestrous stage was found in an earlier study [66]. Recently, O'Byrne and colleagues [93] showed that the reduction of ARC kisspeptin expression by virus-induced *Kiss1*-antisense expression resulted in a decrease in the amplitude but not in the incidence of LH surge in rats. Thus, the ARC kisspeptin neurons may play some roles in the generation and/or amplification of GnRH/LH surges in rats.

Extra-hypothalamic Kisspeptins

Emerging evidence indicates the potential involvement of extra-hypothalamic kisspeptin in reproductive functions. Table 1 summarizes the localization and potential roles of kisspeptin located outside the hypothalamus.

Kisspeptin neurons in the limbic system and hippocampus

Kisspeptin neurons have been found in the medial amygdala and the bed nucleus of stria terminalis, a limbic system [59, 64, 94]. In addition, *Kiss1* expression was also found in the rat hippocampus at lower levels than in the two major hypothalamic populations or in the amygdala [95]. Limbic and hippocampal *Kiss1* expression seems to be controlled by sex steroids: Estrogen increases *Kiss1* expression in the limbic system in rats and mice of both sexes [64, 94], whereas androgen decreases *Kiss1* expression in male rat hippocampus [96]. In the medial amygdala, *Kiss1* expression shows sexual dimorphism with males having more kisspeptin neurons than females [94]. Compared to the hypothalamic kisspeptin neurons, little is known about the physiological role of kisspeptin neurons in the limbic system and hippocampus. Recently, Pineda *et al*. [97] showed reciprocal connectivity between the accessory olfactory bulb and the amygdala kisspeptin neurons, suggesting the role of amygdala kisspeptin neurons as putative mediators of olfactory control of the reproductive function in rodents. Indeed, Comninos *et al*. [98] and Gresham *et al*. [99] showed that injection of kisspeptin into the amygdala enhanced LH secretion in rats, suggesting that kisspeptin-GPR54 signaling in the amygdala may have a physiological role in stimulating LH secretion in rats.

Ovarian kisspeptin

Kisspeptin expression has been found in the ovary of rats [100, 101], but the results are inconsistent between these studies. Castellano *et al*. [100] showed kisspeptin-immunoreactivities in the theca layers of growing follicles, the corpora lutea, and the interstitial tissue of the rat ovary. In contrast, Laoharatchatathanin *et al*. [101] showed *Kiss1* mRNA almost solely in the follicles of rat ovary using the laser-capture microdissection technique. Both studies showed a transient increase in *Kiss1* mRNA at the proestrus or after human chorionic gonadotropin (hCG) stimulation. Ovarian *Kiss1* expression seems to be controlled by preovulatory LH surge [100] and a kisspeptin antagonist exerts a negative influence on the shape of the corpus luteum *in vivo* and progesterone production from granulosa cells *in vitro* [101]. These suggest that ovarian kisspeptin may serve as a local regulator of luteinization. On the other hand, Ricu *et al*. [102] suggested that ovarian kisspeptin acts as a local regulator of follicular development, because local administration of the same kisspeptin antagonist at a higher dose exerts a negative influence on puberty onset and estrous cyclicity without changes in plasma LH levels in rats. Recently, two groups showed that ovarian *Kiss1* expression is higher in aged rats and mice than in young ones and suggested a possible role of ovarian kisspeptin in reproductive senescence, in particular ovarian aging [103, 104].

Recently, forced ovulation in *Kiss1* or *Gpr54* knockout mice was reportedly achieved by a combination of estradiol priming and a standard superovulation protocol using equine CG and hCG [105]. In addition, the oocytes collected from *Kiss1* knockout mice were successfully fertilized with wildtype mouse sperm and developed to the blastocyst stage *in vivo*. This suggests that ovarian kisspeptin is dispensable for oocyte maturation. Further studies are warranted to clarify the role(s) of local kisspeptin in oocyte fertilizability and developmental ability.

Testicular kisspeptin

There are conflicting results on the *Kiss1*/kisspeptin expression in the testis. Mei *et al*. [106] found *Kiss1* or *Gpr54* promoter-driven β-galactosidase activity in the testis as well as in the hypothalamus of knock-in mice carrying targeted mutations in *Kiss1* or *Gpr54* loci with a *LacZ* insertion. More specifically, the β-galactosidase activity was almost solely found in haploid spermatids. The same study failed to detect any kisspeptin-immunoreactivities in spermatids, suggesting that the *Kiss1* mRNA may be translationally repressed in mice.

Pinto *et al*. [107] reported that kisspeptin- and GPR54-immunoreactivities were found in mature human spermatozoa and suggested that kisspeptin-GPR54 signaling may control sperm motility and hyperactivation. So far, there is little physiological evidence regarding the function of the sperm, such as motility and fertility. The functions of the sperm should be analyzed in *Kiss1* or *Gpr54* knockout animal models to resolve this conflict.

Placental and uterine kisspeptin: an implication to implantation

Kisspeptin was first identified in human placenta [35, 36], because it is highly expressed in the syncytiotrophoblast cells of the placenta [108, 109] and its concentrations in the maternal blood markedly increased throughout the period of pregnancy [108, 110]. In rat placenta, kisspeptin expression is found in the trophoblast giant cells and transiently increases at embryonic day 12 when the embryo

Table 1. Localization and potential roles of extra-hypothalamic kisspeptin

Brain area/Organ	Species	Potential roles of kisspeptin
Limbic system	Rats [64, 94] and mice [59, 94]	Mediator of olfactory function [97]
		Control of LH secretion [98, 99]
Hippocampus	Rats [95, 96]	Cognition and epilepsy [96]
Ovary	Rats [100−103] and mice [104]	Corpus luteum formation and progesterone production [101]
		Follicular development [102]
		Ovarian aging [103, 104]
Testis	Mice [106] and humans [107]	Sperm motility and hyperactivation [107]
Uteri	Mice [111]	Implantation [105]
Placenta	Rats [37] and humans [35, 36, 108, 109]	Implantation [37]

Reference numbers are shown in square brackets.

exhibits the implantation [37]. This suggests that kisspeptin may be involved in the implantation in rodents, though the physiological role(s) of large amounts of placental kisspeptin is presently unknown even in humans.

Kiss1 and *Gpr54* expression is also found in uteri in mice. Zhang *et al*. [111] showed that both *Kiss1* and *Gpr54* expression increases with the initiation of implantation and the progression of uterine decidualization. Calder *et al*. [105] showed that *Kiss1* heterozygous embryos failed to implant in superovulated *Kiss1* knockout mice. Thus, uterine kisspeptin is likely involved in the implantation process. In addition, Calder *et al*. [105] showed insufficient expression of leukemia inhibitory factor, a cytokine absolutely required for implantation in mice [112], and the administration of leukemia inhibitory factor to superovulated *Kiss1* knockout females was sufficient to partially rescue the implantation of *Kiss1* heterozygous embryos. These studies may indicate a novel role of uterine kisspeptin in embryonic implantation.

Conclusion and Unanswered Questions

Ever since the discovery of kisspeptin in 2001, intensive studies on hypothalamic expression of *KISS1*/*Kiss1* and on physiological roles of hypothalamic kisspeptin neurons have provided a clue as to how the brain controls sexual maturation at the onset of puberty and subsequent reproductive performance in mammals. As described in this review, the two major populations of hypothalamic kisspeptin neurons are considered centers generating GnRH pulses and surges. There are still some important unanswered questions on hypothalamic kisspeptin neurons. First, it remains unclear how the ARC kisspeptin neurons are synchronized to each other in order to generate the pulsatile kisspeptin and hence GnRH secretion. Morphologically, the fibers of the kisspeptin neurons extend over the whole ARC, indicating a neuronal connection among kisspeptin neurons. Second, afferent inputs to the ARC kisspeptin neurons remain unsettled. In particular, inhibitory inputs to the ARC kisspeptin neurons responsible for physiological restriction of GnRH/gonadotropin secretion during the prepubertal and lactation periods are still poorly understood. Our recent study demonstrates that kisspeptin neurons integrate the stimulatory inputs of glutamatergic and noradrenergic neurons to stimulate GnRH secretion in rats [54]. Thus, such stimulatory inputs may be suppressed or inhibited during the prepubertal and lactation periods, and besides, particular inhibitory inputs to kisspeptin neurons, if any, may mediate GnRH/gonadotropin suppression under the adversity.

In contrast to the intra-hypothalamic roles of kisspeptin, little is known about the physiological significance of kisspeptin produced in the extra-hypothalamic tissues as mentioned in the last part of the present review. So far, the results obtained from the *Kiss1* or *Gpr54* knockout animal models demonstrated that a central defect of *Kiss1* expression accounts for a large portion of the hypogonadotropic hypogonadism. These results, however, cannot eliminate the possibility that extra-hypothalamic kisspeptin serves as an autocrine/paracrine factor in order to exert its physiological role in the peripheral tissues. Further investigation is needed in order to uncover the peripheral mechanisms controlling reproduction in mammals, in which kisspeptin plays a role.

Acknowledgments

We wish to thank Dr Nicola Skoulding for editorial assistance. The present study was supported in part by grants from the Ito Foundation (to YU); the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (to KM); and by JSPS KAKENHI Grant Numbers 15K18769 (to PV), 26252046 (to HT), 15H05782 and 24380157 (to KM).

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