Kisspeptin: A Central Regulator of Reproduction in Mammals

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Abstract

The discovery of the role of kisspeptin neurons in the regulation of mammalian reproduction in 2003 was one of the biggest breakthroughs in reproductive endocrinology within the last few decades. Research during the past two decades since the discovery of kisspeptin has been unveiling the mechanism of how the hypothalamic kisspeptin neurons control reproductive functions through regulation of gonadotropin-releasing hormone (GnRH) secretion. This article aims to overview kisspeptin research, including the most recent studies from ours and other research groups, and to discuss the possibility of new strategies to control reproductive functions in mammals. In the first section, we introduce the critical role of kisspeptin neurons in puberty onset and reproductive functions in mammals, including the regulation of two modes of GnRH/gonadotropin secretion, namely pulsatile and surge modes. The next section focuses more on the mechanism of how the kisspeptin neurons in the arcuate nucleus in the hypothalamus precisely controls GnRH pulse using other two neuropeptides, neurokinin B and dynorphin A. The article also discusses the mechanism suppressing reproductive function during lactation and other stress conditions through inhibition of kisspeptin neurons and consequent GnRH/gonadotropin secretion, to provide insights on the possibility of new strategies to improve reproductive performance in mammals including domestic farm animals.

Keywords: Anteroventral Periventricular Nucleus, Arcuate Nucleus, Fertility, Gonadotropins, Pre-optic Area.

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Introduction

The regulatory mechanism of gonadotropin-releasing hormone (GnRH) secretion, including positive and negative feedback mechanisms from ovarian steroids to the hypothalamic GnRH secretion, had been a “black box” since 1971 when GnRH was first identified in the hypothalamus of sheep (Burgus et al., 1972) and pigs (Schally et al., 1971) as a regulator of gonadotropins secretion from the pituitary. For the discovery of GnRH, the Nobel Prize was awarded to Dr. Guillemin and Schally in 1977. Forty years later, the discovery of the role of kisspeptin neurons in regulation of mammalian reproduction brought the biggest breakthrough in the study of reproductive neuroendocrinology (de Roux et al., 2003, Seminara et al., 2003). Recent research achievements on a regulatory role of kisspeptin on GnRH secretion are leading us to accumulate knowledge serving for the development of new strategies to control reproductive performance of livestock.

Kisspeptin is encoded by Kiss1, a gene first identified as a metastasis-suppressor gene, and was discovered as a natural ligand of an orphan receptor, GPR54 (Kotani et al., 2001, Ohtaki et al., 2001). Kisspeptin neuro-peptide was originally named “metastin” based on its metastasis-suppressing function of the gene (Ohtaki et al., 2001). These days, this neuro-peptide is often called “kisspeptin” in the field of reproduction. In 2003, two independent research groups in the United States and France demonstrated that loss-of-function mutations of GPR54 cause severe hypogonadotropic hypogonadism and subsequent loss of reproductive function in humans and mice (de Roux et al., 2003, Seminara et al., 2003). These studies greatly contributed to highlight the function of the kisspeptin-GPR54 signaling as a master regulator of reproduction via direct stimulation of GnRH and subsequent gonadotropin release in mammals.

This article aims to overview kisspeptin research during the past two decades, including most recent studies from our research group, and to discuss the possibility of new strategies to control reproductive functions in domestic farm animals.

Control of pulse and surge modes of GnRH/gonadotropin secretion by kisspeptin neurons

The gonadal function is regulated by the hypothalamo-pituitary-gonadal axis (HPG axis) in mammals including rodents, ruminants, and primates. GnRH secretion from the hypothalamus stimulates the secretion of gonadotropins from the pituitary, subsequently enhancing gonadal activities. GnRH is secreted in two different modes, namely in a pulsatile mode at the basal level and a surge mode characterized by a transient and larger amount of secretion, in females (Fig. 1). The timing of each GnRH pulse precisely synchronizes with a luteinizing hormone (LH) pulse, as detected in the pituitary portal blood in ewes (Moenter et al., 1992). The pulsatile secretion of GnRH is critical to maintain the responsiveness of the pituitary to GnRH itself, because chronic exposure to GnRH causes reduction of the GnRH receptor in the gonadotrophs in the anterior pituitary (Nett et al., 1981). Indeed, the secretion of gonadotropins is attenuated after prolonged and constant exposure of GnRH (Belchetz et al., 1978). It is also well known that GnRH/LH
secretion is suppressed by negative feedback from ovarian estrogen while the follicles develop (Bronson, 1981, Evans et al., 1994). The GnRH/gonadotropin pulses at a physiological frequency enhance follicular development in the ovary, leading to an increased circulating estrogen level. The high level of circulating estrogen, in-turn, positively feedbacks to the hypothalamus and induces surge-mode secretion of GnRH/LH, and subsequently induces ovulation.

Fig. 1. Regulation of GnRH/gonadotropin surge and GnRH pulses by kisspeptin.

GnRH pulses from the hypothalamus enhance secretion from gonadotropins. Estrogen from the ovary at this stage negatively feedbacks to kisspeptin neurons in the ARC, so that GnRH/gonadotropins secretions are maintained at a low level. Continuous GnRH/gonadotropin pulses at the basal level are critically important for the follicular growth, which causes higher and higher concentration of estrogen in the blood circulation. When follicular growth reaches to the final stage, a high level of blood estrogen positively feedbacks to the kisspeptin neurons in the AVPV/POA that stimulate GnRH neurons. This leads to the substantial secretion of gonadotropins from the pituitary and subsequently induces ovulation.
G-protein-coupled receptor 54 (GPR54; a kisspeptin receptor) signaling is the fundamental regulator of the HPG axis in mammals as described above. To demonstrate that kisspeptin itself is essential for reproduction, we previously generated Kiss1 knockout (KO) rats, of which the Kiss1 locus was replaced with a red fluorescent protein reporter gene (Uenoyama et al., 2015). The Kiss1 KO rats showed non-detectable levels of plasma gonadotropins, atrophic gonads, and therefore, the absence of spontaneous puberty in both sexes. Further, the lack of kisspeptin caused attenuation of LH pulses as well as the estrogen-induced LH surge (Uenoyama et al., 2015), but an intravenous challenge of kisspeptin robustly enhanced LH secretion in Kiss1 KO rats, leading to the conclusion that kisspeptin is an indispensable stimulator of GnRH/gonadotropin secretion both in the pulse and surge modes.

Kisspeptin neurons are mainly located in the hypothalamic area in two populations: the posterior population of the neurons in the arcuate nucleus (ARC); and the anterior one in the anteroventral periventricular nucleus (AVPV), periventricular nucleus (PeN), or preoptic areas (POA) in females of rodents (Gottsch et al., 2004, Adachi et al., 2007), pigs (Tomikawa et al., 2010), and ruminants (Franceschini et al., 2006, Matsuda et al., 2015, Hassaneen et al., 2016), respectively. Kiss1 gene expression in these neurons is regulated by estrogen, negatively in the ARC and positively in the AVPV, via the estrogen receptor α in kisspeptin neurons of both nuclei in rodents (Smith et al., 2005, Adachi et al., 2007). A hypothesis has been developed over the past few decades suggesting that the GnRH pulse generator locates in the mediobasal hypothalamus including the ARC (Ohkura et al., 1991, 1992), while the GnRH surge generator locates in the AVPV/POA (Goodman, 1978, Petersen and Barraclough, 1989). Given this hypothesis, the notion that kisspeptin neurons in the two hypothalamic nuclei would be the regulators of the two modes of GnRH/gonadotropin secretion is well-accepted by many researchers. Indeed, rhythmic volleys of multiple unit activity (MUA) were found to be synchronized with LH pulses in female goats when the electrode was placed in the close proximity to the ARC kisspeptin neurons (Ohkura et al., 2009), and there was significant correlation between the LH pulse frequency and the number of Kiss1-expressing neurons in the ARC of maturing ewe (Redmond et al., 2011). On the other hand, when a kisspeptin antibody was infused to the POA, an adjacent brain region to the AVPV, endogenous LH surge was attenuated in female rats (Kinoshita et al., 2005). Therefore, it is plausible that ARC kisspeptin neurons are the central regulator of GnRH pulses, whereas the AVPV kisspeptin neurons are the central regulator of the GnRH surge.

As discussed above, AVPV kisspeptin neurons are considered to be a target of positive feedback of estrogen in rodents (Smith et al., 2005, Adachi et al., 2007). Similarly in pigs, kisspeptin neurons in the PeN, equivalent to rodent AVPV kisspeptin neurons, appears to be the center of the GnRH/gonadotropin surge generation because Kiss1 expression in the region increases upon estrogen treatment in ovariectomized sows (Tomikawa et al., 2010). Likewise, in goats, monkeys and musk shrew, an increase in c-Fos, a marker
of neuronal activation, expression in the POA kisspeptin neurons is evident after the administration of preovulatory levels of estrogen (Inoue et al., 2011, Watanabe et al., 2014, Matsuda et al., 2015). Interestingly, in sheep, it is likely that both the ARC and POA kisspeptin neurons take part in GnRH surge generation, because Kiss1 expression in both regions significantly increased during the late follicular phase compared to the luteal phase (Smith et al., 2009). Besides, administration of progesterone, but not estrogen, increases the expression of Kiss1 in the ARC in ewes in breeding season (Smith et al., 2007). Such differences among species should be taken into account in considering the mechanism that controls GnRH/LH surge through kisspeptin neurons in livestock.

Accumulating evidence also suggests the absolute necessity of kisspeptin in the onset of puberty. For instance, the Kiss1-expression level is significantly higher in the post-pubertal period compared to pre-pubertal period in the hypothalamus of rats (Takase et al., 2009), ruminants (Redmond et al., 2011), and primates (Shahab et al., 2005). It is considered that Kiss1-expression in pre-pubertal period is suppressed by the negative feedback of estrogen, because ovariectomy causes an increase in ARC Kiss1-expression as well as LH pulses in immature rats (Takase et al., 2009) and ewes (Nestor et al., 2012). Besides, kisspeptin administration induces LH secretion in prepubertal rats (Navarro et al., 2005) and primates (Shahab et al., 2005). These studies suggest that the HPG axis is already functional in terms of responsiveness of GnRH neurons to kisspeptin and that of gonadotrophs to GnRH even before puberty, and puberty occurs when the hypothalamic Kiss1 is ready to express and secrete kisspeptin to induce GnRH and consequent gonadotropins secretion. It is considered that Kiss1 expression is suppressed by the negative feedback of estrogen more sensitively in the pre-pubertal period than in the post-pubertal period, because a low level of circulating estrogen, which is equivalent to the diestrous level in matured animals, can robustly suppress Kiss1 expression in immature rats (Takase et al., 2009). Therefore, investigation on the mechanism of desensitization of the kisspeptin neurons to the estrogen negative feedback could be a key to understand the precise mechanism of the onset of puberty in mammals.

**GnRH pulse generation by KNDy neurons**

The mechanism responsible for GnRH pulse generation could be shared by wide range of mammalian species. This common mechanism among the species may serve for development of new schemes of reproductive control in livestock. Thus, the mechanism by which ARC kisspeptin neurons control pulsatile GnRH/gonadotropin release will be discussed in this section.

The ARC kisspeptin neurons are reported to co-express two other neuropeptides, namely neurokinin B and dynorphin A, in rodents and ruminants (Goodman et al., 2007, Navarro et al., 2009, Wakabayashi et al., 2010), and therefore are often referred to as kisspeptin/neurokinin B/dynorphin A (KNDy) neurons, whereas these neuropeptides were not found in the AVPV kisspeptin neurons. As mentioned above, MUA volleys synchronizes with LH pulses when the electronic detector is placed in
close adjacent to the KNDy neurons in the ARC of female goats (Ohkura et al., 2009, Wakabayashi et al., 2010). Importantly, such MUA volleys were not observed when an electronic detector was placed in the lateral part of the median eminence, where GnRH neuronal axons project to (Ohkura et al., 2009). Therefore, it is considered that the pulsatile secretion of GnRH and gonadotropins are induced by the pulsatile activity of the ARC KNDy neurons. Interestingly, the frequency of MUA volley of KNDy neurons dramatically increases by a central administration of neurokinin B or a dynorphin A receptor antagonist in the goat whereas the volley is robustly suppressed by the dynorphin A injection (Wakabayashi et al., 2010). These results suggest that KNDy neurons regulate their own activity by autocrine and/or paracrine actions of neurokinin B and dynorphin A to determine the frequency of GnRH pulses in mammals.

Notably, a neurokinin B receptor agonist or a dynorphin A receptor antagonist induces precocious puberty in rats (Nakahara et al., 2013), and loss-of-function mutations in genes encoding neurokinin B or the neurokinin B receptor cause severe hypogonadotropic hypogonadism in humans (Topaloglu et al., 2009) similar to loss-of-function mutations in Kiss1 (Uenoyama et al., 2015) or GPR54 encoding the kisspeptin receptor (de Roux et al., 2003, Seminara et al., 2003). These studies strongly suggest that neurokinin B positively regulates the pulsatile secretion of GnRH in mammals. On the other hand, the precise mechanism of inhibitory regulator of GnRH pulses needs further investigation. The co-expression rate of the dynorphin A in ARC kisspeptin neurons is consistent among species at 80-90% in mice, sheep, and goats (Goodman et al., 2007, Navarro et al., 2009, Wakabayashi et al., 2010), while the co-expression rate of the dynorphin A receptor (kappa-opioid receptor; KOR) in the kisspeptin neurons varies among species and has only been reported in limited number of studies (Navarro et al., 2009, Weems et al., 2016). For instance, a previous study reported that KOR is expressed only in approximately 20% of the ARC kisspeptin neurons in mice (Navarro et al., 2009), while 98% of ARC kisspeptin neurons showed KOR immunoreactivity in ewe (Weems et al., 2016). Further studies on the precise localization of each receptor of the neuropeptides are expected to contribute to drawing a clear picture of the neuronal circuit of the GnRH pulse generator.

Our previous study demonstrated a part of the mechanism of how the KNDy neurons synchronize their activity and cause pulsatile firing as a group, by using Kiss1-GFP transgenic mice (Ikegami et al., 2017). In the study, we demonstrated that senktide (a selective agonist for neurokinin B receptor) induced Ca^{2+} oscillations, an indicator of neuronal activity, in cultured Kiss1-GFP cells were synchronized amongst themselves, as well as with those in neighboring glial cells (Ikegami et al., 2017). In addition, our study suggested that the cell-to-cell communication through gap junctions between Kiss1-GFP cells as well as Kiss1-GFP cells and neighboring glial cells is involved in synchronized activities among KNDy neurons in order to generate GnRH pulses. Considering also that among the ARC kisspeptin neurons, more than 90% express neurokinin B and the neurokinin B receptor in mice and goats (Navarro et al., 2009, Wakabayashi et al., 2010), at least neurokinin B signaling would be a stimulatory regulator of GnRH
pulse generation via autocrine manner among KNDy neurons.

The recently emerged concept that kisspeptin neurons regulate GnRH pulses using neurokinin B and dynorphin A signaling may serve for the development of new strategies and/or drugs to control reproduction in farm animals. We recently succeeded to facilitate pulsatile LH section by a single peripheral shot of a dynorphin A receptor antagonist to female goats (Sasaki et al., 2019). The regimen would be also applicable to cattle, one of the most economically important farm animals in many countries, because our study showed the presence of KNDy neurons in cows (Hassaneen et al., 2016) and a neurokinin 3 receptor-selective agonist accelerated pulsatile luteinizing hormone secretion in lactating cattle (Nakamura et al., 2017). More specific focus on the importance of controlling reproductive function in milking cows will be discussed in the following section. In addition, a mechanism mediating malfunction of reproduction caused by heat stress and malnutrition in mammals and possibility of new methods for treatment of the reproductive disorder will also be discussed.

**Inhibition of GnRH/gonadotropin secretion during lactation and other stress conditions**

Mammalian reproductive function is inhibited during lactation and other stress conditions such as malnutrition and heat stress, which are major causes of economic loss in both developed and developing countries. In mammals, a tremendous amount of energy is required to produce milk in lactating females. Therefore, to avoid pregnancy that requires extra energy expenditure, follicular development and ovulation are strongly suppressed during lactation (McNeilly, 2001). It has been pointed out that the conception rate and milk yields are negatively correlated in genetically improved dairy cows and that the suppressed reproductive performance may be caused by high energy expenditure required for milk production (Nebel and McGilliard, 1993). In addition, in developing countries in tropical or arid areas, extremely hot climates and a lack of food supply may cause heat-stress and malnutrition, respectively. The inhibition of reproductive function in mammals often relies on the central suppression of pulsatile GnRH and gonadotropin secretion, and the mechanisms of GnRH suppression under certain conditions such as lactation and stress, have been elucidated by recent studies. In this section, each mechanism responsible for reproductive suppression by lactation, malnutrition, or heat stress will be discussed.

The inhibition of reproductive function during lactation is at least partly caused by suckling stimulus-induced neuronal suppression on GnRH/gonadotropins pulses, because the hypothalamic deafferentation to isolate the mediobasal hypothalamus restores LH pulses in lactating mother rats (Tsukamura et al., 1990). Our group has previously demonstrated that Kiss1 expression was suppressed by suckling stimulus in rats, while LH release was immediately restored by an administration of kisspeptin to the mother rat (Yamada et al., 2007). Interestingly, estrogen positive feedback to the hypothalamus is functional during lactation, because the LH surge can be induced by administration of estrogen at a proestrous level (Tsukamura et al., 1988)
and LH pulses were restored 12 hours after the removal of the suckling stimulus in lactating rats (Maeda et al., 1989). These findings have led us to consider that the inhibition of GnRH/LH pulses by suckling stimulus in lactating animals is mainly caused by suppression of kisspeptin production in the ARC. Further, our most recent study suggested that somatostatin signaling at least partly mediates LH pulse suppression in lactating rats (Sugimoto et al., 2019).

It is well known that malnutrition also suppresses the reproductive function in mammals. Precise analysis on plasma hormones in rodents revealed that LH pulses are strongly suppressed in fasted animals (Cagampang et al., 1990, Minabe et al., 2011). Undernourished heifers showed longer estrous cycles, poor corpus luteum formation with significantly lower plasma progesterone concentration, and reduced number or unusual development of follicles, compared to those fed with a sufficient diet (Hill et al., 1970). Leptin is a hormone mainly secreted from the adipocytes (Zhang et al., 1994) and its plasma concentration drops along with body weight loss in humans (Weigle et al., 1997), and with food restriction as in ewes (Recabarren et al., 2004). Leptin is known to affect the HPG axis via neuropeptide Y and pro-opiomelanocortin neurons in the ARC (Cunningham et al., 1999) and restores gonadotropin secretion in fasted rats (Nagatani et al., 1998) and monkeys (Finn et al., 1998). Neuropeptide Y, an orexigenic neuropeptide inhibits estrogen-induced LH surge through neuropeptide Y type 2 receptor (Clarke et al., 2005), and melanocortins, a group of anorexigenic neuropeptides produced from pro-opiomelanocortin, are mainly reported to enhance the LH surge (Backholer et al., 2009), as both studies demonstrated in ewes. Interestingly, in ob/ob mice, a mutant strain in where leptin is deficient, ARC Kiss1 expression is partially impaired but restored by leptin administration (Smith et al., 2006). In ewes, it is also reported that leptin increased the expression level of Kiss1 in the ARC (Backholer et al., 2010). It was demonstrated that the kisspeptin neurons interact with their adjacent neurons expressing neuropeptide Y and pro-opiomelanocortin neurons, supporting the notion that kisspeptin neurons may be involved in gating the fasting-induced suppression of reproduction (Backholer et al., 2010, Fu and van den Pol, 2010). The mRNA expression of Ob-Rb, a gene encoding the leptin receptor, is detected in the Kiss1-expressing neurons in the ARC in mice (Smith et al., 2006). It is also possible that kisspeptin neurons are under regulation by the anorexigenic neurons, because administration of melanocortin receptors agonist accelerated GnRH generator activity detected as MUA volleys in goats (Matsuyama et al., 2005).

We have previously proposed that negative energy balance of the body can be sensed by the hindbrain to inhibit GnRH/LH secretion, based on the studies demonstrating that central injections of 2-deoxy-D-glucose (a glucose metabolic inhibitor) (Murahashi et al., 1996), alloxan (an inhibitor of glucokinase, a rate-limiting enzyme for glucose metabolism) (Kinoshita et al., 2004), ketone body (by-product of enhanced fatty acid mobilization) (Iwata et al., 2011), mercaptoacetate or trimetazidine (inhibitors of fatty acid oxidization) (Sajapitak et al., 2008), or AICAR (an
inhibitor of AMP-activated protein kinase; AMPK) into the fourth ventricle in the hindbrain suppress pulsatile LH secretion in rats. It has been also shown that glucokinase, glucose transporters, and phosphorylated AMPK are evident in the ependymocytes surrounding the ventricle of the hindbrain (Maekawa et al., 2000, Minabe et al., 2015), and that the intracellular calcium concentration in the ependymocytes taken from the hindbrain increases in response to administration of a low or high level of glucose (Moriyama et al., 2004) or AICAR (Minabe et al., 2015) in the cultured medium. Our most recent study demonstrated that the ependymocytes of the fourth ventricle have neuronal connection to hypothalamic kisspeptin neurons (Deura et al., 2019). The study also implicated the possibility that the noradrenergic neurons and corticotropin-releasing hormone neurons mediate a part of the pathway from the hindbrain to the hypothalamus (Deura et al., 2019). Precise investigation on the direct neuronal inputs into the kisspeptin neurons will be the key to obtain complete understanding of the mechanism how malnutrition is sensed by the brain to suppress GnRH/gonadotropins secretion.

Cows subject to hot weather are known to display a lower frequency of LH pulses compared to those under a more controlled and cooler environment (Wise et al., 1988). Previous research demonstrated that LH pulse amplitude was decreased in animals that had lower plasma estradiol concentration which were under heat stress (Gilad et al., 1993). It should be clarified if the heat stress suppresses gonadotropin release through affecting KNDDy neurons. Other types of stress such as restraint stress are reported to suppress cfos expression in the ARC Kiss1 neurons while corticosterone secretion is enhanced in mice (Yang et al., 2017). This suppression could be caused by increased dynorphin A signaling in the ARC, because the promoter activity of Pdyn, a gene encoding dynorphin A, is enhanced by presence of the glucocorticoid receptor agonist in a cell line derived from mice hypothalamus (Ayrout et al., 2019). The link between body-temperature control and KNDDy neurons has also been implicated in a previous study, which demonstrated that female rats with a functional ablation of KNDDy neurons, induced by a selective neurotoxin for neurokinin B receptor-expressing cells, showed the lower and higher core body temperature compared to control animals under high (33°C) and low (11°C) ambient temperature, respectively (Mittelman-Smith et al., 2012). Collectively, considering those studies, we note the possibility that KNDDy neurons may play a role in suppression of GnRH/LH pulses as well as body temperature control under heat stress.

Suppression of GnRH/LH pulses during lactation and under stress is a critical mechanism to ensure efficient use of energy and maximize the survival of an individual, and their offspring. On the other hand, in the context of livestock industry, these periods when GnRH/LH pulses, and then reproductive performance are suppressed are often a bottle neck of productivity in the field. Since kisspeptin neurons play an essential role to inhibit or enhance the reproductive function in mammals, kisspeptin and related peptides, such as neurokinin B and dynorphin A, can be the key molecules to unlock the development of innovative method to control reproductive function in livestock.
For instance, a possible novel method to treat the reproductive disorders would be the administration of neurokinin B agonists and/or dynorphin A antagonists. Development of these drugs for veterinary use would be necessary to enable peripheral administration of the drugs as well as maximizing their efficacy.

**Conclusion**

The discovery of kisspeptin at the beginning of this century contributed greatly to the progress in uncovering the central mechanism of the regulation of mammalian reproduction. Especially, the KNDy neurons in the ARC, where GnRH pulse generating mechanism have long been hypothesized to locate, may be responsible for the pulsatile secretion of GnRH/gonadotropin. This notion has attracted great attention among researchers in the field of mammalian reproduction. Further studies on regulatory factors on KNDy neuronal activities and the mechanisms would greatly contribute to the innovative technologies to enhance reproductive function in livestock.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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