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Kisspeptin (kiss 1) network signaling of hypothalamic gonadotropin-releasing hormone (GnRH) neurons

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ABSTRACT

To regulate reproduction in mammals, internal and external status is communicated to the hypothalamic-pituitary-gonadal axis *via* a coordinated balance of stimulatory and inhibitory neurochemical systems. This review describes the neuroanatomical organization of the kisspeptin system in the hypothalamus, and summarizes its role in signaling hypothalamic GnRH neurons, namely: multimodal actions of kisspeptin in the control of GnRH secretion during different physiological states of animals; involvement of kisspeptin in relaying metabolic information to hypothalamic GnRH neurons; neurobiological mechanisms underlying kisspeptin activation of GnRH neurons during puberty onset; effect of gonadal steroids in the early postnatal period in rodents on kisspeptin expression in the hypothalamus and gonadotropin secretion in adults; effect of photoperiod and metabolic cues on the activation/inhibition of hypothalamic-pituitary-gonadal axis in seasonally breeding species by kisspeptin.

KEY WORDS: hypothalamus, kisspeptin neurons, GnRH, rodents, sheep, primates

INTRODUCTION

Kisspeptin 54 (termed kiss 1) has recently emerged as a key player in the regulation of many physiological functions, among which the most recognized is

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elicitation of GnRH secretion (Seminaria et al., 2003; Smith et al., 2006c).

Compelling experimental evidence has strongly suggested the involvement of kisspeptin in many physiological processes of reproduction, including positive and negative feedback of sex steroids on gonadotropin secretion (Terasawa et al., 2010), generation of the preovulatory GnRH surge (Lehman et al., 2010), metabolic regulation of fertility (Roa et al., 2008), photoperiodic control of reproduction in seasonal breeders (Chalivoix et al., 2010), maturation and timing of puberty onset (Amstalden et al., 2010 b; Clarkson et al., 2010), sex and species-specific differences of kisspeptin neurons in signaling hypothalamic GnRH cells (Homma et al., 2009; Kauffman et al., 2009), the effect of gonadal steroids in early postnatal life in rodents on kisspeptin expression in the hypothalamus and gonadotropin secretion in adult females and males (Kauffman et al., 2009).

In rodents, kisspeptin expression in the hypothalamus and gonadotropin secretion in adult females and males are affected by gonadal steriods acting in the early postnatal period.

In a wide range of mammals, such as mice, rats, sheep, pigs and primates, kiss 1 mRNA or kisspeptin protein have been detected mainly in two distinct regions within the hypothalamus: the first is in the preoptic area, which in rodents constitutes a morphological continuum comprising the anteroventral hypothalamus (AVPV) region, the second is the more caudally situated arcuate nucleus (ARC) (Oakley et al., 2009). In rodents, two discrete subpopulations of kiss 1 neurons with different functional properties in response to oestrogens exist in the ARC and AVPV. The neurons of kisspeptin in the ARC have been implicated in the negative feedback action of oestrogens, while AVPV kisspeptin cells in female rodents may participate in the positive feedback action of gonadal steroids (Kauffman et al., 2009) (Figure 1).

Kisspeptin, encoded by the kiss 1 gene, is a high-affinity peptide ligand (West et al., 1998) for the orphan G-protein-coupled membrane receptor (GPR 54) called kiss 1 r (Ohtaki et al., 2001). The initial product of the kiss 1 gene (Oakley et al., 2009) is a 145-amino acid protein that is enzymatically cleaved into a 54-amino acid peptide, known as kiss 1. There are also shorter peptides (kisspeptin 10, 13, 14 amino acids) that share the RF amidated motif of kiss 1 (Figure 1). It is likely that kiss 1 is unstable and proteolyticaly cleaves into these shorter products (Kotani et al., 2001). However, all four peptides exhibit the same affinity and efficiency for kiss 1r, indicating that the C terminal end of the peptides is responsible for the binding and activation of kiss 1 r. Although all of these kisspeptin products are biologically active, the *in vivo* relevance of the shorter forms is less recognized (Oakley et al., 2009). The binding of kisspeptin to kiss 1r leads to a cascade of reactions, from the activation of G protein phosphatase C (PLCB), synthesis of intracellular second messengers, inositol triphosphate (IP3) and diacylglycerol

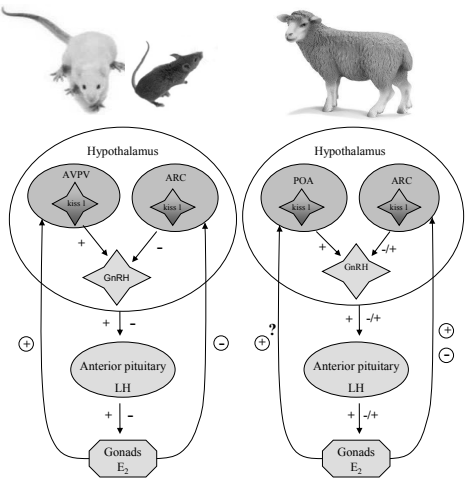

Figure 1. The schematic comparison of positive and negative feedback inputs into the hypothalamic-pituitary-gonadal axis through kisspeptin (kiss 1) neurons in the anteroventral periventricular nucleus (AVPV), arcuate nucleus (ARC) and preoptic area (POA) within the hypothalamus of rodents and sheep. GnRH - gonadotropin releasing hormone, LH - luteinizing hormone, E_2 - oestradiol, + - stimulation, – - inhibition, ⊕ - positive feedback action, ⊖ - negative feedback action

(DAG), to intracellular Ca^{++} release and activation of protein kinase C (PKC) (Oakley et al., 2009). Kisspeptin stimulates GnRH secretion through a phospholipase C/calcium-dependent pathway regulating multiple ion channels (Zhang et al., 2008).

The kiss 1 neurons in many discrete nuclei of the hypothalamus and other brain regions, as well as outside the central nervous system (CNS) varies across species (Lee et al., 2009; Oakley et al., 2009). In the murine hypothalamus, kiss 1 mRNA and kiss-immunoreactive cell bodies are expressed mostly in the AVPV, periventricular nucleus (PeV), ARC, and dorsomedial hypothalamus (DMH) (Clarkson et al., 2009; Oakley et al., 2009). In addition, cells expressing kiss 1 mRNA are located in the preoptic area (POA) and bed nucleus of stria terminals (BNST) (Oakley et al., 2009). Although the distribution of kisspeptin cells is similar between males and females, there are significant sex differences in the number of cell bodies in the AVPV/PeV. The adult female mouse exhibits about 10-fold more kisspeptin immunoreactive cells than do males (Messager et al., 2005; Oakley et al., 2009).

In the rat hypothalamus, kiss 1 cells are found in the AVPV/PeV, ARC, DMH, paraventricular nuclei (PV), and ventromedial hypothalamus (VMH). Most of the kisspeptin cells in this species are located in the DMH, but cells containing kiss 1

mRNA have not been detected in this structure by in *situ* hybridization (Oakley et al., 2009). Similarly, antibodies do not label kisspeptin neurons in the AVPV/PeV, in which high expression of kiss 1 mRNA has been established (Oakley et al., 2009). In adult female rats, the AVPV contains about 25 times more cells that express kiss 1 mRNA compared with males (Kauffman et al., 2007a; Oakley et al., 2009). The hypothalamic GnRH cells in mice and rats also co-express kiss 1 mRNA (Irwing et al., 2004; Oakley et al., 2009; Clarkson et al., 2010).

In the Syrian hamster, a rodent with ovarian cycles during a long-day photoperiod, such a photoperiod increases kisspeptin in the ARC (Roseweir et al., 2009). Levels of kiss 1 mRNA in the ARC are reduced in male Syrian hamsters after transfer from long-day to short-day conditions, which leads to reproductive quiescence (Revel et al., 2007). Chronic infusion of kisspeptin restores testicular activity in this species despite persistence of photoinhibitory conditions (Revel et al., 2006). On the other hand, in Siberian hamsters, kisspeptin and kiss 1 mRNA expression increase in the ARC and decrease in the AVPV after transfer to a short photoperiod (Paul et al., 2009b). Both male and female Siberian hamsters held under short-day conditions exhibit a reduced response to exogenous kisspeptin treatment and show negligible kisspeptin expression in the AVPV and high expression in the ARC (Mason et al., 2007). Under long-day conditions, the expression of kiss 1 is increased in the AVPV and a only minor elevation is observed in the ARC (Greives et al., 2007; Mason et al., 2007).

In sheep, a short-day breeding species, most kisspeptin immunoreactive cells are detected in the ARC, DMH, POA, VMH, and in the caudal part of the PV (Pompolo et al., 2006; Oakley et al., 2009). Kiss 1 mRNA expression in the ARC is lower during seasonal anoestrus than during the breeding season (Smith et al., 2008 b). There also exist sex differences in the number of kisspeptin neurons in the ovine hypothalamus: adult rams have nearly half the number of kisspeptin neurons in the ARC as do females (Cheng et al., 2010). In contrast to rodents, the GnRH cells in the hypothalamus of sheep do not express kisspeptin (Oakley et al., 2009).

MULTIMODAL ACTION OF KISSPEPTIN NEURONS IN THE HYPOTHALAMUS ON GNRH CELLS: FACTORS AFFECTING KISSPEPTIN NEURON ACTIVITY

The control of kisspeptin neuron activity comes from a variety of sources, including steroid hormones (Oakley et al., 2009), peptides (Backholer et al., 2010a,b), photoperiodic cues (Chalivoix et al., 2010), metabolic signals (Fernandez-Fernandez et al., 2006), and others (Plant et al., 2006; Kauffman et al., 2009; Oakley et al., 2009).

Some influences of kisspeptin on reproductive processes are highly conserved across species, such as involvement of kisspeptin neurons in the negative feedback action of oestrogen on GnRH release (Oakley et al., 2009). Other aspects of oestradiol on GnRH secretion through kisspeptin systems in different hypothalamic structures are unique to particular species and gender, like the stimulatory action of oestrogen on GnRH release in females (Oakley et al., 2009) or its influence in the early postnatal period in females and males on kisspeptin neuron expression in adulthood (Kauffman et al., 2009). Similarly, kisspeptin neurons react in a specific way to the duration of light in seasonally breeding animals (Greives et al., 2007; Revel et al., 2007). Generally, the level of kisspeptin in these animals is higher during the breeding season compared with the non-breeding anoestrous period. For example, in long-day seasonal breeders like the Syrian hamster, the expression of kiss 1 mRNA in the ARC rises in the long-day photoperiod, while in ewes, short-day breeding animals, its increased expression in this area occurs during the seasonal short day (Smith et al., 2007; Oakley et al., 2009).

Kisspeptin neurons in the AVPV of rodents appear to play a central role in the positive feedback effect of oestradiol on GnRH cells. Indeed, nearly all kisspeptin neurons in this structure of female rodents express oestradiol receptors and treatment with oestradiol causes significant increases in the expression of kiss 1 mRNA (Oakley et al., 2009) in them. The expression of kiss 1 mRNA in the AVPV peaks at a time coincident with the GnRH/LH surge, and kisspeptin neurons in this structure show fos induction at the time of gonadotropin output (Smith et al., 2006a; Oakley et al., 2009). The positive feedback effect of oestradiol through the kisspeptin system in the AVPV on preovulatory release of GnRH is also suggested by other lines of evidence. Kisspeptin neurons from this area make direct synaptic contact with GnRH cells and treatment with kisspeptin antiserum completely abolishes the GnRH surge (Oakley et al., 2009; Pineda et al., 2010). The transient suppression of kisspeptin signaling prevents the occurrence of the primary surges of LH and FSH, and the secondary peak of FSH (Pineda et al., 2010) in rats. Nonetheless, despite the inhibition of hormonal surges, basal LH and FSH levels are not overtly decreased by administration of an antagonist of kisspeptin in cyclic female rats (Pineda et al., 2010).

Recent studies indicate that kisspeptin neurons also represent an important component of the negative feedback loop of oestradiol on GnRH release. This function is carried out by a subpopulation of kisspeptin neurons located mainly in the ARC (Navarro et al., 2004). The expression of kiss 1 mRNA in the ARC of rats changes throughout the oestrous cycle, with levels reaching a nadir at/or around the time when the oestradiol levels are highest (Smith et al., 2006a). On the other hand, a decrease in blood oestradiol concentrations after ovariectomy causes a rise in kiss 1 mRNA in the ARC of rodents, sheep, and monkeys (Navarro et al., 2004; Smith et al., 2007; Kim et al., 2009).

In sheep, kisspeptin neurons have been found mainly in the ARC and POA (Oakley et al., 2009) and all cells in the ARC display both positive and negative feedback responses to oestradiol (Felip et al., 2009; Smith et al., 2009a), whereas in the POA, kiss is positively involved in the feedback action of oestradiol on GnRH/LH secretion (Smith et al., 2009a) (Figure 2). The positive feedback effect of oestradiol on GnRH neurons in sheep is mediated by kisspeptin neurons in the middle to caudal parts of the ARC, as revealed by up-regulation of kiss 1 mRNA during the periovulatory period (Smith et al., 2008b). Indeed, kisspeptin cells

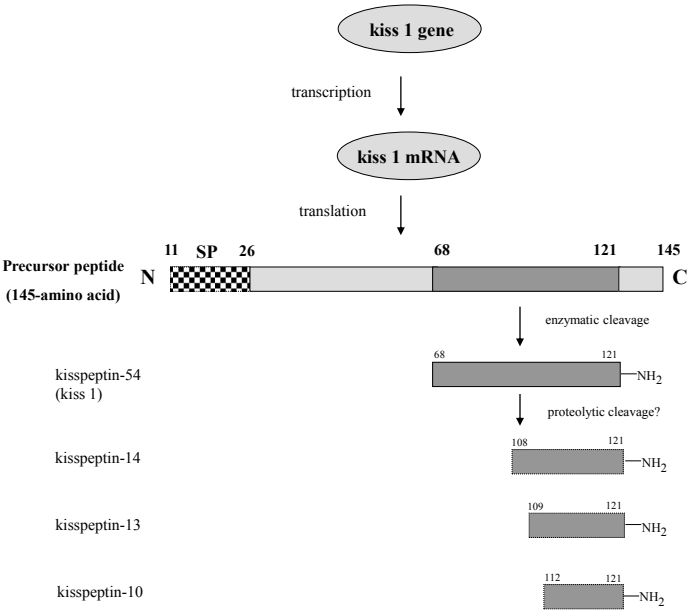

Figure 2. Schematic diagram showing posttranscriptional and posttranslational product of kiss 1 gene - 145-amino-acid precursor peptide, and relative cleavage points of precursor peptide that lead to the synthesis of kisspeptin-54 (named as kiss 1) and shorter peptides such as kisspeptin-10, -13, and -14). SP - signal peptide, N - amino-terminal domain, C - carboxyl-terminal domain

within this region of sheep became transcriptionally activated after GnRH surge-inducing oestradiol stimulus, and kisspeptin protein fos expression and kiss 1 mRNA were greater immediately prior to the GnRH surge (Smith et al., 2008b, 2009a), thus suggesting a stimulatory role of kisspeptin in oestrogen-positive feedback on GnRH secretion. On the other hand, the increase of GnRH/LH secretion in ewes after ovariectomy with a concomitant rise in kiss 1 mRNA expression in the ARC indicates that at least the same subpopulation of kisspeptin cells in this area mediates the inhibitory effect of oestradiol on the activity of GnRH neurons (Smith et al., 2010).

Recent studies evidenced that kisspeptin cells in the POA of ewes may also be involved in the positive feedback effect of oestrogen on GnRH secretion and preovulatory GnRH/LH surge. The increase of POA kiss mRNA in the follicular phase in ewes suggests that the POA kisspeptin cell subpopulation may also contribute to the stimulation of GnRH secretion during the surge (Smith et al., 2009b). It is suggested that in female sheep, the induction of GnRH release and preovulatory GnRH/LH surge involves the sequential action of oestradiol on the ARC+POA kisspeptin cell populations (Lehman et al., 2010). It is possible that the early effect of oestrogen within the ARC is to sensitize the POA kisspeptin system for subsequent triggering of the GnRH surge (Caraty et al., 2010; Cheng et al., 2010).

Several lines of evidence indicate that kisspeptin signals directly to the GnRH system: the majority of GnRH cells in rodents express kiss 1 and kiss 1r (Oakley et al., 2009; Herbison et al., 2010), and kisspeptin fibres are closely associated with GnRH neurons (Clarkson et al., 2007). Kisspeptin can act directly to depolarize and increase firing rates of GnRH cells *in vitro* (Zhang et al., 2008) or through synaptic mechanism(s) (Oakley et al., 2009) to stimulate GnRH secretion. Kisspeptin neurons in the median eminence can affect GnRH cells in a nonsynaptic manner (Smith et al., 2007; Ramaswamy et al., 2008). The precise molecular mechanisms by which oestradiol differentially regulates kisspeptin cell activity is unknown. Oestrogen receptors can exert a multiplicity of cellular effects in kisspeptin neurons depending upon their interplay with different signaling pathways.

The classical pathways involves oestradiol binding to the gene promoter of oestrogen-sensitive neurons to alter transcription (Oakley et al., 2009). An alternative oestrogen receptor signaling pathway operates through a nonclassical mechanism with protein-protein interactions (Glidewell-Kenney et al., 2007; Gottsch et al., 2009). Analysis of these interactions in female mice indicates that the positive effect of oestradiol on the expression of kiss 1 is mediated by the classical pathway in the AVPV and by the nonclassical negative feedback regulation of LH release in the ARC (Gottsch et al., 2009; Oakley et al., 2009). These results show that kisspeptin neurons in the AVPV and ARC of rodents respond in a site-specific manner to the action of oestrogen on GnRH (Gottsch et al., 2009). The role of AVPV kisspeptidergic afferents in the generation of GnRH release should be viewed with caution, however, because opposite results of the persistence of the positive feedback of oestradiol have been reported in different mouse lines with a null mutation of kisspeptin receptors (Oakley et al., 2009). In studies based on models of congenital absence of kisspeptin or kisspeptin receptors, it has been shown that GnRH/LH secretion persisted in many animals in which kisspeptin signaling was inactivated (Chan et al., 2009a,b), whereas in other models, inactivation led to complete reproductive failure (Clarkson et al., 2007; Dungan et al., 2007).

There is still a lack of fundamental understanding of how kisspeptin neurons of the ARC in ewes mediate both negative and positive feedback of oestradiol on GnRH secretion. Indeed, oestradiol inhibits the kisspeptin-induced rise in LH secretion in mice and rats, and blocks the postcastration LH increase in rodents and sheep, suggesting a major role of kisspeptin neurons of this structure in mediating the negative feedback action of sex steroids on the hypothalamic-pituitary-gonadal axis activity (Roseweir et al., 2009, 2010). Both the positive and negative feedback action of oestradiol evidenced in ewes raises questions how the same set of neurons can have both stimulatory and inhibitory effects on GnRH secretion in different physiological states. The new likely explanations are that subsets of neurons in the ARC of ewes mediate different actions of oestradiol or that the same subset of neurons responds differentially to low and high concentrations of steroids, perhaps through different intracellular signaling mechanisms. It is likely that oestradiol in the ARC acts *via* multiple pathways to induce the preovulatory GnRH surge or to inhibit basal GnRH secretion, one of these pathways may be through ARC kisspeptin/neurokinin B/dynorphin (KNDy) neurons (Lehman et al., 2010).

Very recently, the availability of the first factor with the ability to block kisspeptin action *in vivo* and *in vitro*, kisspeptin antagonist p 234, has made it possible to characterize in detail the role of endogenous kisspeptin in the control of GnRH secretion. It has been shown that acute, transient suppression of kisspeptin signaling prevents the occurrence of a GnRH/gonadotropin surge, but does not alter basal LH and FSH secretion in rats. Blockade of kisspeptin receptors suppresses both GnRH and oestradiol-induced gonadotropin surges and increases LH secretion after gonadectomy, but does not affect basal secretion of these hormones in rats (Pineda et al., 2010).

The inhibition of LH and FSH surges by a kisspeptin antagonist without affecting basal gonadotropin secretion may be useful in designing therapeutic approaches in reproduction by selectively targeting surges without abolishing basal gonadotropin secretion (Pineda et al., 2010).

In the ARC, a subpopulation of ovine kisspeptin neurons expresses dynorphin and neurokinin B (Goodman et al., 2007; Cheng et al., 2010). The co-localization of KNDy peptide cells in the ARC is unique among brain regions and is conserved across multiple mammalian species that include rats, mice, goats, and humans (Lehman et al., 2010). A large body of evidence strongly suggests that KNDy cells and individual KNDy peptides can influence the activity of GnRH cells by acting directly at the level of their cell bodies in the POA and/or their neurosecretory terminals in the median eminence (Foradori et al., 2006; Lehman et al., 2010). This does not preclude also the possibility that projections of KNDy cells to interneurons, such dynorphin or kisspeptin neurons in the POA, play a major role

in the control of GnRH release (Gottsch et al., 2009; Lehman et al., 2010).

Evidence in rats (Ciofi et al., 2006) and sheep (Billings et al., 2010) indicates that KNDy cells and their terminals colocalize with the vesicular glutamate transporter-2 (vGLUT-2), suggesting that they are glutaminergic as well as peptidergic in phenotype (Wu et al., 2009; Lehman et al., 2010). Colocalization of glutamate with kisspeptin cells and their projections onto GnRH neurons in the ewe suggest that co-release of kisspeptin and glutamate from ARC neurons may plays a physiological role in steroid feedback control of GnRH secretion (Lehman et al., 2010). The majority of vGLUT-2 positive cells in the ARC of the sheep are colocalized with oestrogen α receptors and provide a role for glutamate in conveying the feedback influence of oestradiol during the preovulatory GnRH surge (Lehman et al., 2010). It is suggested that the coordinated release of kisspeptin and glutamate provides dual stimulatory signals to activate either the KNDy subpopulation or GnRH neurons during the follicular phase of the oestrous cycle in ewes (Lehman et al., 2010). This point of view is supported by the observation that in the ARC of mice, N-methyl D-aspartic acid (NMDA), an NMDA receptor (NMDAR) agonist, causes an increase of c-fos expression in kisspeptin cells, thus suggesting the kisspeptin-dependent action of NMDA stimulating GnRH release (D'Anglemont de Tasigny et al., 2010). Although there is evidence that NMDAR are expressed on GnRH cell bodies as well as on GnRH terminals at the level of the median eminence, it is not known whether KNDy cells express these or other glutamate receptor subtypes (Lehman et al., 2010).

The ARC KNDy neurons also have receptors for dynorphin and neurokin B (NKB) (Navarro et al., 2009; Amstalden et al., 2010a), thus suggesting the existence of autosynaptic contact among KNDy; kisspeptin neurons generate pulses of kisspeptin release, which in turn drives pulsatile GnRH secretion (Tovar et al., 2006). It is possible that kisspeptin cells exert both a stimulatory and inhibitory influence on themselves and on another cells of this type. Indeed, peripheral administration of kisspeptin elicits GnRH/LH pulses in rats and monkeys (Plant and Witchel, 2006; Tovar et al., 2006). Continuous delivery of exogenous kisspeptin can desensitize kiss 1, resulting in decreased LH secretion (Seminaria et al., 2006). This implies that the efficiency of kisspeptin on gonadotropin secretion depends on the pulsatile manner of its release. Interestingly, sustained kisspeptin treatment in seasonally anoestrous ewes increased LH secretion and caused ovulation in most animals (Caraty et al., 2007). It is likely that the endogenous kisspeptin in the ARC may be inhibited by oestradiol during negative feedback, and stimulated during positive feedback, which in turn may alter synaptic transmission to GnRH neurons.

Dynorphin A, an opioid peptide, is also a critical mediator in the negative feedback of progesterone in GnRH secretion (Cheng et al., 2010). In ewes

dynorphin appears to act as an inhibitory factor on pulsatile GnRH secretion and mediates progesterone negative feedback on gonadotropin release during the luteal phase of the oestrous cycle (Lehman et al., 2010).

Similarly, the NKB is implicated in steroid feedback control of GnRH secretion, although its aspect may be species-specific; it has indirect stimulatory action on GnRH neurons in sheep and humans (Billings et al., 2010; Topaloglu et al., 2010), whereas in rats and mice its displays a rather suppressive influence on the secretion of this hormone (Corander et al., 2010; Lehman et al., 2010). Single ARC KNDy cell groups in ewes co-express both stimulatory and inhibitory neuropeptides, making alterations in the levels or release of each a potential mechanism for feedback control of GnRH neuron activity.

Kisspeptin increases gamma-aminobutyric acid (GABA)-ergic transmission directly or indirectly to GnRH neurons in an oestradiol-dependent manner and can increase GnRH neuron response to activation of GABA receptors in rats (Zhang et al., 2009; Pielecka-Fortuna and Moenter, 2010).

There is morphological evidence for the existence of an interrelationship between kisspeptin- and nitric-oxide-containing neurons in the control of GnRH secretion (Bellefontain et al., 2011). Preliminary data show that kisspeptin- and nitric-oxide-containing neurons stimulate GnRH cells directly or indirectly *via* action on synaptic afferents to GnRH neurons. Additionally, kisspeptin neurons may also talk to neuronal nitric oxide synthase (nNOS) neurons and promote nitric oxide (NO) production that could serve as an intermediately synchronizing 'switch' of GnRH secretion (Bellefontain et al., 2011). Recent studies have demonstrated that intracerebroventricular injection of NMDA induces nNOS neuron activation and LH release in kisspeptin- and kisspeptin receptor-knockout mice (Bellefontain et al., 2011)

Although kisspeptin does not participate directly in the control of metabolic function and energy expenditure (Backholer et al., 2010b, Donato et al., 2011), there is growing evidence that the hypothalamic kisspeptin system plays a key role in conveying metabolic information to GnRH neurons (Roa et al., 2008; George et al., 2011). A critical amount of energy reserve is necessary for maintenance of cyclicity and fertility in females in most cases. Ovulation is usually suppressed when animals are in negative energy balance induced by different events (Brown et al., 2008; Donato et al., 2011). In adult mice subjected to short-term fasting, there is a rapid decline in both kiss 1 and kiss 1r followed by a decrease in GnRH secretion (Levis et al., 2002). It has been reported that kiss 1 mRNA is reduced in both prepubertal and adult male and female food-deprived rats (Roa et al., 2008, 2010). The levels of kisspeptin receptors in the ARC and POA are lower in hypogonadotropic lean animals than in animals of normal weight (Castellano et al., 2005; Backholer et al., 2010b). In the state of undernutrition, which reduces

gonadotropin secretion, kisspeptin administration can reinstate reproductive function (Navarro et al., 2005b; Castellano et al., 2009).

Numerous orexigenic and anorectic peptides produced in the central nervous system and/or peripheral tissue are involved in the fine regulation of feeding and reproduction by kisspeptin neurons in the hypothalamus. The adipocyte-derived hormone, leptin, which signals the size of body energy stores, is likely to play a pivotal role in the kisspeptin system regulating reproductive function (Backholer et al., 2010b; Donato et al., 2011). In the mouse, kisspeptin cells in the ARC and POA express the signaling form of leptin receptor (Navarro et al., 2005a; Smith et al., 2006b) and leptin is able to act directly on the kisspeptin system or indirectly *via* modulation of afferent pathways to kisspeptin cells such as proopiomelanocortin/neuropeptide Y (POMC/NPY) neurons (Backholer et al., 2010b; Roa et al., 2010). Leptin is able to normalize kiss 1 gene expression in models of impaired gonadotropin secretion linked to hypoleptinaemia (Donato et al., 2011).

Kisspeptin neurons in the ovine ARC and POA respond to leptin; expression of kiss 1 mRNA to in response to leptin is affected by the physiological state (Backholer et al., 2010b). In lean, but not in normal animals, a decrease in kiss 1 gene expression, as well as putative secretion of LH, can be partially restored by leptin treatment (Backholer et al., 2010b). Additionally, leptin acts to down-regulate NPY gene expression in animals of normal weights, but not in lean animals (Ahima and Hileman, 2000; Backholer et al., 2010b). Leptin administration to leptin-deficient (ob/ob) or leptin-receptor-deficient (db/db) subjects induces puberty and fertility (Donato et al., 2011). Leptin levels fall during starvation and leptin administration blunts the fasting-induced suppression of LH and restores fertility (Donato et al., 2011).

It was shown that the POMC system within the brain is also an important factor in the regulation of energy balance and regulation of reproductive processes in many species. Using double-label fluorescent immune-histochemistry, a complex reciprocal connection between kisspeptin cells in the POA and ARC with POMC/NPY cells has been documented (Backholer et al., 2009, 2010a). An important concept is that the kisspeptin system affects POMC/NPY neurons by altering their gene expression, allowing a means by which kisspeptin cells may modulate appetite-regulating neurons (Backholer et al., 2010a, 2010b) and indirectly control GnRH secretion (Kim et al., 2010). The kisspeptin reduces POMC and increases NPY gene expression (Backholer et al., 2010b). NPY displays an inhibitory effect on GnRH/LH release and a stimulatory one on food intake (Clarke et al., 2005). Similarly to kisspeptin, leptin also positively regulates POMC gene expression in rodents (Thornton et al., 1997; Donato et al., 2011). Leptin administration in the hypogonadotropic state of lean ovariectomized ewes up-regulates POMC gene

expression in lean ewes, partially restoring kiss 1 mRNA expression in these animals, it is possible that transmission of leptin to GnRH cells predominantly occurs through melanocortin- but not kisspeptin systems (Backholer et al., 2010a). Indeed, activation of melanocortin receptors in lean animals restores LH levels, suggesting that down-regulation of the POMC system is the cause of the hypogonadotropic condition (Donato et al., 2011). Conclusive experimental evidence has been presented recently, showing that under physiological conditions, the actions of leptin in the control of GnRH function are indirect and likely conducted *via* the modulation of afferent pathways (Quennel et al., 2009).

TThe POMC prohormone is post-translationally cleaved to produce β-endorphin and melanocortins, α-melanocyte-stimulating hormone (α-MSH), β and γ-MSH (Backholer et al., 2009). Endogenous β-endorphin negatively regulates reproduction and stimulates food intake (Backholer et al., 2009), on the other hand, melanocortins reduce food intake (Watanabe et al., 1999; Backholer et al., 2009) and stimulate gonadotropin secretion acting *via* their respective receptors (Backholer et al., 2010a)

The orexigenic gut peptide, ghrelin, which plays a major role during negative energy balance to maintain whole-body energy homeostasis (Agata et al., 2009; Briggs and Andrews, 2011), also provides feedback information on metabolic status to kisspeptin neurons. It could directly or indirectly suppress GnRH/LH acting *via* opioid peptides, NPY, corticotropin-releasing hormone (CRH), and kisspeptin neurons under conditions of deficient nutrition (Agata et al., 2009; Forbes et al., 2009; Roa et al., 2010). Fragmentary results suggest that ghrelin negatively modulates the activity of the hypothalamic-pituitary-gonadal axis in rats; fasting, ghrelin treatment, or their combination, down-regulate kiss 1 expression in the POA and decrease pulsatile LH secretion (Kurose et al., 2005; Agata et al., 2009; Forbes et al., 2009; Roa et al., 2010). Ghrelin levels are reduced in lean ovariectomized ewes (Kurose et al., 2005), but it is unknown whether this affects kisspeptin expression in this species.

EFFECTS OF GONADAL STEROIDS IN THE EARLY POSTNATAL PERIOD IN RODENTS ON KISSPEPTIN EXPRESSION IN THE HYPOTHALAMUS AND ON GONADOTROPIN SECRETION

Kisspeptin in the hypothalamus has been recently recognized as an important regulator of GnRH neurons both in development and adulthood (Oakley et al., 2009). Numerous studies on different species have provided results supporting the mechanism(s) of kisspeptin neuron action *via* kiss 1r on GnRH cells (Kauffman et al., 2009). These works have documented that in rodents, kiss 1 gene expression in the AVPV is up-regulated by high levels of oestrogen; in the absence of gonadal

steroids after gonadectomy in adult females, kiss 1 levels in this structure are decreased, leading to disappearance of the GnRH/LH surge (Smith et al., 2006a; Kauffman et al., 2009). Similarly, pharmacological or transgenic blockade of kisspeptin signaling prevents the preovulatory gonadotropin surge (Kinoshita et al., 2005; Pineda et al., 2010). Kisspeptin neurons in the AVPV of rodents are sexually differentiated; adult females have greater kisspeptin expression than males (Kauffman et al., 2007b). The sex differences in AVPV kiss 1 expression in adulthood likely account for the sex-specific ability of female, but not male, rodents, to produce a gonadotropin surge (Kauffman et al., 2009). Indeed, the number of kisspeptin neurons in the AVPV correlates with the ability or inability of an animal to generate an LH surge: adult females have high kiss 1 levels and can generate a GnRH/LH surge, whereas adult males have little AVPV kiss 1 expression and cannot produce a gonadotropin surge, even with oestradiol treatment (Kauffman et al., 2009).

This sexually-dimorphic reproductive physiology of gonadotropin secretion has been demonstrated to be differentiated during the perinatal action of sex steroids (Corbier, 1985). Female newborn rats do not normally secrete significant levels of gonadal hormones during the perinatal 'critical period'; the absence of high levels of circulating sex steroids in perinatal females results in their brain kiss 1 being differentiated to feminized (Handa and Gorski, 1985; Kauffman et al., 2009) - leading to the ability of these animals to generate a GnRH/LH surge in adulthood. Female newborn rats exposed to oestradiol or testosterone display masculinized levels of kisspeptin immunoreactivity in the AVPV in adulthood and cannot generate a GnRH/LH surge in response to sex steroid treatment (Bateman and Palisaul, 2008). They possess very few kisspeptin neurons in the AVPV as adults, similarly to normal adult males (Kauffman et al., 2007a, 2009) and show male-like levels of kiss 1 mRNA (Handa and Gorski, 1985).

In contrast to females, during the perinatal period of development male rats secrete testosterone, which induces masculinization of kisspeptin neurons in the AVPV; this is reflected in the low expression of kiss 1 mRNA in adults (Homma et al., 2009). Newborn rats castrated on the day of birth have high kiss 1 levels in the AVPV as adults (Homma et al., 2009), indicating that the AVPV kiss 1 system sexually differentiates under the influence of postnatal gonadal steroids (Homma et al., 2009; Kauffman et al., 2009). After oestradiol treatment, these rats can generate an LH surge similarly as females (Kauffman et al., 2009).

Sex differences in ARC kisspeptin neurons that mediate oestradiol negative-feedback regulation of GnRH secretion, which manifests during peripubertal development, are absent in adult rats and mice (Kauffman et al., 2007b, 2009); adult rodent females and males display similar high levels of kisspeptin expressionin in the ARC after gonadectomy and similar reduced kisspeptin expression after steroid

replacement (Homma et al., 2009; Kauffman et al., 2009). Intact prepubertal male and female mice, however, express low levels of kisspeptin in the ARC, whereas kisspeptin levels significantly increase in gonadectomized females but not in males (Kauffman et al., 2009). The increased kisspeptin expression in ovariectomized early postnatal life of female mice indicates that there is no gonadal hormone-independent suppression of ARC kisspeptin neurons in prepubertal female mice and suggests that the prepubertal female reproductive axis appears to be kept quiescent predominantly by gonadal hormone negative feedback (Oakley et al., 2009; Cheng et al., 2010). In contrast, in prepubertal males, ARC kiss 1 expression 2-4 days following gonadectomy was similar to that in intact males (Homma et al., 2009; Kauffman et al., 2009). This lack of increased kiss 1 expression after gonadectomy in prepubertal males shows that some non-gonadal mechanism(s) suppress ARC kisspeptin neurons in these males. Since adult mice of both sexes exhibit significant increases in ARC kiss 1 expression, this phenomenon indicates that sex differences in gonadal hormone-independent suppression of the ARC kisspeptin system exists only during the short period of prepubertal development. Also, prepubertal LH levels display the same sexually-dimorphic pattern as ARC kiss 1 expression in gonadectomized prepubertal females and males (Kauffman et al., 2009). Thus, the regulation of GnRH/LH secretion during prepubertal development is sexually dimorphic.

Although gonadectomized prepubertal male mice did not exhibit short-term elevation of LH secretion and ARC kiss 1 expression, they increased ARC kiss 1 mRNA and LH levels in adulthood (Kauffman et al., 2009). Similarly as in male mice, a gonadal hormone-independent mechanism(s) has developed in primates to control gonadotropin secretion and puberty onset (Plant, 2006; Plant et al., 2006). Increased NKB and LH in response to gonadectomy in both sexes have been demonstrated in postpubertal female, but not male, mice. This suggests that gonadal hormone-independent mechanism(s) restrain kiss/NKB-associated activation of the male reproductive axis before puberty (Kauffman et al., 2007b). This is consistent with data from primates showing that gonadectomized juvenile monkeys do not display elevated LH before the normal age of puberty (Plant and Witchel, 2006) and also from human males with gonadal disgenesis (Topaloglu et al., 2010). The precise mechanism(s) of the action of the non-gonadal inhibitory factors involved in this process remains unknown, however.

In sheep, both the positive and negative feedback action of oestradiol on GnRH/LH secretion is mediated by kiss 1 neurons in the ARC (Smith et al., 2008b). Recent studies on kiss 1 expression in adult male and female sheep have indicated that females possess a greater number of kisspeptin neurons in the ARC than males (Cheng et al., 2010). Prenatal testosterone treatment reduces dynorphin/neurokinin B (DYN/NKB) expression in the KNDy cells in the ARC but not the number

B (DYN/NKB) expression in the KNDy cells in the ARC, but not the number of kisspeptin neurons, suggesting that multiple neuropeptides expressed by the same neurons may have different 'critical periods' for sexual differentiation and/or that the kisspeptin system of the ovine ARC is not sexually differentiated by prenatal sex steroid treatment (Cheng et al., 2010). The imbalance of neuropeptide DYN/NKB expression within KNDy cells by prenatal testosterone treatment provides a potential explanation for defects in gonadal hormone feedback control of GnRH secretion seen in ewes (Clarke et al., 1997; Robinson et al., 1999). The basis for species differences in kisspeptin systems in the prenatal-neonatal period in response to oestradiol is not entirely known, however, but may be connected with species differences in the neural region mediating sex steroid feedback on GnRH secretion. In rodents, the positive feedback of oestradiol is mediated by the AVPV, whereas in sheep, the ARC is involved in the control of the preovulatory GnRH surge (Foster et al., 2006; Smith et al., 2008b).

EFFECTS OF KISSPEPTIN ON PITUITARY GONADOTROPES

In respect to the direct action of kisspeptin on gonadotropes, conflicting findings have been reported in mammals. Several studies have demonstrated that kisspeptin stimulates gonadotropin release *in vitro* from cultured rat, ovine, and bovine primary pituitary cells, suggesting that kisspeptin may act directly on gonadotropes to stimulate LH and FSH secretion (Muir et al., 2001; Tovar et al., 2006; Oakley et al., 2009). Kiss 1 and kiss 1r genes are expressed in rat gonadotropes and differentially regulated by oestradiol and GnRH (Richard et al., 2008). Kiss 1r mRNA is expressed in ovine pituitary cell fractions enriched for gonadotropes, which allows the possibility that kisspeptin is either a hypophysiotropic/neurosecretory factor in sheep or there are paracrine mechanisms involving kiss 1r signaling within the pituitary (Smith et al., 2008a). By contrast, other studies have shown no apparent effect of kisspeptin on *in vitro* gonadotropin secretion in cultured rat primary pituitary cells or pituitary fragments (Kotani et al., 2001; Ohtaki et al., 2001). Similarly, in *in vivo* experiments the direct effect of kisspeptin on pituitary gonadotropes failed to stimulate gonadotropin secretion in hypothalamic-pituitary disconnected ewes during steady-state conditions (Smith et al., 2008b). Whether gonadotropes are targets for kisspeptin action remains still unresolved in mammals.

INVOLVEMENT OF THE HYPOTHALAMIC KISSPEPTIN SYSTEM IN THE ONSET OF PUBERTY

Studies undertaken in many species indicate that kisspeptin-kisspeptin receptor signaling at the level of GnRH neurons is essential for normal puberty onset

(Messager et al., 2005). They have identified the three most important features leading to the onset of puberty: the increase of kisspeptin receptor mRNA in the GnRH neurons of hypothalamus; a modest increase in the electrical response of GnRH neurons to kiss 1r activation across postnatal development; and the appearance of kisspeptin fibres surrounding GnRH neurons just prior to puberty onset (Clarkson et al., 2010). They also suggest that the key step in kisspeptin control of puberty is the regulation of kisspeptin synthesis within the kisspeptin cell populations located in the rostral periventricular region of the third ventricle (RP3V) in rats and mice (Clarkson et al., 2010). More specifically, the involvement of kisspeptin signaling in the timing of reproductive puberty onset was originally suggested on the basis of observation of the lack of puberty linked to congenital deficiency of kiss 1r (Oakley et al., 2009). In humans and mice, mutation or lack of kiss 1r results in failure to undergo puberty (Chan et al., 2009b; Roseweir et al., 2009). Similarly, mice lacking the kiss 1 gene exhibit absence of reproductive development (Thompson et al., 2004).

A marked increase in kiss 1 and/or kiss 1r expression is associated with the onset of puberty (Castellano et al., 2006; Amstalden et al., 2010b). It has been reported that the number of kisspeptin neurons in the AVPV of mice increases exponentially from the early postnatal period through puberty (Clarkson et al., 2010). The percentage of kiss 1r mRNA does not differ between juvenile and adult animals, however (Han et al., 2005). During this period the percentage of GnRH neurons responding to kisspeptin increases approximately from 25 to more than 90% in adults, suggesting that GnRH neurons become more sensitive to kisspeptin during postnatal development, and that kisspeptin neuron activity increases with increasing oestradiol secretion in growing female rats (Han et al., 2005). In intact female monkeys, kisspeptin transcript expression is three-fold greater in midpubertal stages compared with juvenile or early pubertal animals, corresponding to the progressive increase in kiss 1r mRNA from juvenile to midpubertal stages (Shahab et al., 2005). An increase of kisspeptin pulses was also observed at the time of puberty (Keen et al., 2008). In the monkey, the kisspeptin neurons in the ARC may be a source of the GnRH pulse generator, showing nearly coincident secretion of kiss 1 and GnRH (Keen et al., 2008). Altogether, these findings seem to provide strong support for an important role of the kisspeptin system in the hypothalamus in mediating puberty (Oakley et al., 2009). Such a statement should be viewed with caution, however, because the results from mice models of kiss 1 and kiss 1r gene knockouts indicate the existence of a kiss 1/kiss 1r independent GnRH regulatory mechanism(s) (Oakley et al., 2009).

The molecular and cellular mechanisms that lead to the onset of puberty may differ markedly across species and gender (Kauffman et al., 2009). Studies on monkeys indicate that GnRH neurons are already mature before puberty, but GnRH

release is suppressed by tonic GABA activation. Blocking endogenous GABA activity with the GABAA receptor blocker bicuculline, significantly increases kisspeptin release, which plays an important stimulatory role in the prepubertal increase of GnRH release (Terasawa et al., 2010). Thus, the interplay between GABA, kisspeptin and GnRH neural systems appears to trigger puberty onset at least in this species. Blocking the action of kisspeptin by the action of p 234 makes it possible to characterize the timing of puberty: vaginal opening and changes in uterine and ovarian weights at the expected time of puberty with concomitant analysis of gonadotropin secretion. The kisspeptin antagonist inhibits the firing rates of GnRH neurons, GnRH secretion, and suppresses LH responses to exogenous kiss 1 and gonadectomy (Pineda et al., 2010). This suggests that the delay in puberty of animals treated with p 234 might be caused by central inhibition of the afternoon surges of LH that precede the occurrence of vaginal opening (Ojeda and Skinner, 2006; Pineda et al., 2010). The phenotypic effects of gonadotropin cells are not apparently associated with the decrease in mean circulating levels of LH and FSH at the end of antagonist treatment. These observations are in accordance with data from adult rats, where administration of p 234 suppress LH release resulting from gonadectomy, but not basal gonadotropin secretion (Roseweir and Millar, 2009).

Recently, a new model was proposed for the mechanism of ARC KNDy neuron participation and for their action in the control of human reproduction. It is suggested that neurokinin B and dynorphin A act autosynaptically to synchronize pulsatile secretion of kisspeptin in the ARC, which in turn drives the release of GnRH in the median eminence (Foradori et al., 2006). It is still unclear whether the role of NKB in pubertal onset is permissive or whether an increase in NKB is required. In rats and mice, the intraventricular administration of NKB or its synthetic analogue, senktide, decreases LH secretion (Sandoval-Guzman and Rance, 2004; Navarro et al., 2009), whereas in humans and sheep, NKB has a stimulatory effect on GnRH/LH release (Amstalden et al., 2010a; Topaloglu et al., 2010). Interestingly, intraventricular co-administration of NKB and kisspeptin in rats caused a significant increase in LH concentration compared with kiss 1 alone (Corander et al., 2010). Recent studies have demonstrated increased NKB and kisspeptin along with LH response to gonadectomy in both sexes in postpubertal mice. Such changes occur in females but not in males prepubertally, suggesting that gonadal hormone-independent mechanisms restrain kisspeptin/NKB activation of male reproduction before puberty (Kauffman et al., 2007b). Similarly, gonadectomized juvenile monkeys do not display elevated LH before normal puberty nor do human males who have gonadal dysgenesis (Topaloglu et al., 2010).

Kisspeptin is also a major player in the control of puberty onset by metabolic cues. Studies in female rats subjected to chronic caloric restriction during puberty have demonstrated a detectable suppression of kiss 1 expression in the ARC (Castellano et al., 2009; Roa et al., 2010) and pharmacological treatment with kisspeptin at the expected time of puberty was sufficient to rescue vaginal opening and induce a potent gonadotropin response (Roa et al., 2010). Preliminary evidence from postnatal undernutrition of female rats indicates that leptin is an important regulator of kisspeptin expression at the time of puberty: there is a strict correlation between low circulating leptin levels and hypothalamic levels of kiss 1 mRNA and number of kisspeptin neurons (Roa et al., 2010).

Among other possible candidates, ghrelin, as a circulating orexigenic factor that signals energy insufficiency, has emerged as a putative modifier of the timing of puberty (Roa et al., 2010). Indeed, studies involving acute or repeated administration of ghrelin in pubertal rats support the function of ghrelin as a putative modulator of puberty having a predominantly inhibitory action (Forbes et al., 2009; Roa et al., 2010). Ghrelin may operate in the hypothalamus as a putative inhibitory regulator of kisspeptin expression and be able to suppress the kiss 1 gene in the preoptic area in rats (Forbes et al., 2009). A progressive decline in circulating ghrelin levels during puberty in humans suggests that ghrelin may play a permissive role in puberty onset, assuming a similar inhibitory action on gonadotropin secretion (Roa et al., 2010).

EFFECTS OF PHOTOPERIOD AND NUTRITIONAL CUES ON KISSPEPTIN LEVELS IN THE HYPOTHALAMUS AND ON REPRODUCTIVE ACTIVITY IN SEASONALLY BREEDING SPECIES

It is generally accepted that annual cycles of reproduction in sheep and other seasonal breeders are primarily induced by photic-mediated changes in the activity of several neuroendocrine systems and their functions in controlling GnRH secretion (Adams et al., 2006) and GnRH receptor (GnRHR) gene expression (Ciechanowska et al., 2010). The neural mechanisms responsible for transition of photic-signals into GnRH cells are not yet completely elucidated. Recent studies have indicated that seasonally breeding animals use a combination of photic and nonphotic (temperature, food availability) signals for regulation of reproduction (Paul et al., 2009a; Prendergast, 2010). To maximize reproductive success, animals restrict breeding to the optimal time of year, when internal physiology and external environmental conditions are suitable for the survival of both parent and offspring. It has been recognized that melatonin signals, which encode photoperiods, act within the mediobasal hypothalamus in sheep to control GnRH secretion (Malpaux et al., 1998) through neural pathways including dopaminergic (Goodman et al., 2010), noradrenergic (Goodman et al., 1995), serotoninergic

(Le Corre and Chemineau, 1993), opioidergic (Skinner and Herbison, 1997), GABAergic (Scott and Clarke, 1993) and other pathways.

After the discovery of kisspeptins, it was shown that this peptide may also be involved in the seasonal messages of melatonin in generation of annual cycles in reproduction (Maywood et al., 1996).

In the ewe, a short-day breeding species, kiss 1 mRNA in the middle and caudal ARC, which displays a stimulatory effect on GnRH release, is lower during seasonal anoestrus than during the breeding season (Smith et al., 2007). Maximal expression of kiss 1 mRNA in the caudal ARC of sheep was observed during treatment with a photoperiod mimicking short days, and minimal expression, during long-day treatment (Wagner et al., 2008). An increase in kisspeptin neurons was detected in the POA and caudal ARC after transition to a short day (Chalivoix et al., 2010). It is suggested that these differences between kiss 1 mRNA and number of kisspeptin neurons in the POA and ARC may result from an increase in kisspeptin synthesis under short-day conditions. It has also been reported that the photoperiod exerts a considerable influence on the number of kisspeptin opposition on GnRH cells in the sheep hypothalamus with a highest synaptic density during the breeding season (Smith et al., 2007). It is likely that the morphological rearrangements of kisspeptin neurons in the hypothalamus between the breeding and non-breeding period lead to differences in the gonadotropic response to kisspeptin in various functional states of the ewe reproductive axis. Similarly, morphological rearrangements in the *organum vasculosum* of the lamina terminalis (OVLT)/POA neurons and A15 dopaminergic neurons have been observed in adult ewes during different seasons of reproduction (Xiong et al., 1997; Adams et al., 2006).

In most photoperiodic rodents, the short photoperiod, which triggers gonadal regression, usually leads to decreases in kiss 1 mRNA and its respective protein expression in the AVPV (Paul et al., 2009a). Indeed, levels of kiss 1 mRNA in the ARC in male Syrian hamsters are reduced after transfer from long- to short-day conditions, which leads to reproductive quiescence (Revel et al., 2006). These seasonal changes in kiss 1 mRNA appear to be melatonin-dependent, because pineal gland ablation blocks this short-day-induced down-regulation of kisspeptin expression, and melatonin-induced atrophy of the reproductive system (Revel et al., 2006). Chronic infusion of kiss 1 restores testicular activity in Syrian hamsters despite persisting photoinhibitory conditions (Revel et al., 2006).

Both male and female Siberian hamsters held under short-day conditions exhibit reduced responses to exogenous kisspeptin treatment and show negligible kiss 1 expression in the AVPV and its high expression in the ARC (Mason et al., 2007). Under long-day conditions, these expressions are reversed, with a marked level in the AVPV and only a minor one in the ARC (Greives et al., 2007; Mason et al., 2007). This indicates that responses in kisspeptin expression to photoperiod may be species-specific.

A recently developed experimental method whereby nonphotic seasonal regulation of reproduction is investigated under a static photoperiod has provided novel insights into the environmental influence through the kisspeptin system on GnRH/LH secretion (Paul et al., 2009a). It has been shown that modest reduction of food availability decreases kiss 1 mRNA at the level of the ARC of male Siberian hamsters either by down-regulation of kiss 1 transcription or by increasing posttranscriptional processes. These data are consistent with reports in other nonphotoperiodic species (Levis et al., 2002; Smith et al., 2006c). At the level of the AVPV, neither photoperiod nor food restriction affected kiss 1 mRNA expression in this species. Recent studies have shown that another hypothalamic RF amide peptide, RFRP-3, may figure predominantly in transduction of inhibitory signals to the hypothalamic-pituitary-gonadal axis. At the level of DMH, food restriction does not alter RFRP-3 gene expression; exposure of Siberian hamsters to intermediate photoperiod does, however, increase RFRP-3 mRNA expression in this structure. This indicates that nonphotic (reduced food availability) and photic (intermediate day lengths) stimuli are inadequate to impact reproductive physiology. In neither the ARC nor DMH were additive effects of food restriction on physiological events observed. Gonadal responses were only associated with changes in both ARC kiss 1 mRNA and DMH RFRP-3; such a pattern of responses is consistent with a role of the RF amide system as an integrator of multimodal seasonal cues in reproduction. This regional and neuropeptide-specific activity of these systems may provide a mechanism for integration of photic and nonphotic action in the seasonal control of reproduction.

CONCLUSIONS

The accumulated evidence indicates that kisspeptin neurons, located in species-specific areas of the hypothalamus, are involved in the stimulation and/or inhibition of GnRH/LH secretion during different physiological states of animals. The action of kisspeptin neurons on GnRH activity in the hypothalamus is multifactorial and is dependent to a high degree upon steroid hormones, various peptides, and metabolic signals. The kisspeptin-GPR 54 system also participates in the mechanisms of puberty onset, the effect of gonadal steroids in the early postnatal period on the pulsatile pattern of LH secretion in adults, and the influence of photoperiod and metabolic factors on the activity of the hypothalamic-pituitary-gonadal axis in seasonally breeding animals.

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