The involvement of gonadotropin inhibitory hormone and kisspeptin in the metabolic regulation of reproduction

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Abstract

Recently, kisspeptin (KP) and gonadotropin inhibitory hormone (GnIH), two counteracting neuropeptides, have been acknowledged as significant regulators of reproductive function. KP stimulates reproduction while GnIH inhibits it. These two neuropeptides seem to be pivotal for the modulation of reproductive activity in response to internal and external cues. It is well-documented that the current metabolic status of the body is closely linked to its reproductive output. However, how reproductive function is regulated by the body's energy status is less clear. Recent studies have suggested an active participation of hypothalamic KP and GnIH in the modulation of reproductive function according to available metabolic cues. Expression of *KISS1*, the KP encoding gene, is decreased while expression of *RFRP* (*NPVF*), the gene encoding GnIH, is increased in metabolic deficiency conditions. The lower levels of KP, as suggested by a decrease in *KISS1* gene mRNA expression, during metabolic deficiency can be corrected by administration of exogenous KP, which leads to an increase in reproductive hormone levels. Likewise, administration of RF9, a GnIH receptor antagonist, can reverse the inhibitory effect of fasting on testosterone in monkeys. Together, it is likely that the integrated function of both these hypothalamic neuropeptides works as a reproductive output regulator in response to a change in metabolic status. In this review, we have summarized literature from nonprimate and primate studies that demonstrate the involvement of KP and GnIH in the metabolic regulation of reproduction.

Key Words
- kisspeptin
- Kiss1r
- GnIH
- GPR147
- metabolism
- reproduction
- leptin
- ghrelin

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Introduction

Reproduction in nonprimate mammals and in primates is highly responsive to metabolic alterations (Cameron 1991, Wade & Jones 2004). Deficiencies of metabolic fuels prevent the proper release of gonadotropin releasing hormone (GnRH) from the hypothalamus, thus causing reproductive quiescence (Bergendahl *et al*. 1991, Cameron *et al*. 1991, Wahab *et al*. 2013*a*,*b*). Initiation of reproductive function is delayed by conditions of negative energy balance while, in adults, merely skipping one daily meal can lead to reproductive quiescence in nonhuman primates (Kennedy & Mitra 1963*a*, Foster & Olster 1985, Cameron 1991, 1996). Food resumption regularizes the

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dysfunctional reproductive axis and normalizes reproduction (Parfitt *et al*. 1991). However, the exact mechanisms regarding metabolic impact on reproductive output are still poorly understood.

In the last decade, our understanding of the metabolic regulation of reproduction has been greatly enhanced through the detection of different neuroendocrine peptides. Besides many others, these include the two most recently discovered peptide hormones, kisspeptin (KP) and gonadotropin inhibitory hormone (GnIH) (Pinilla *et al*. 2012, Ubuka *et al*. 2013). KP and GnIH are two neuropeptides of the hypothalamus. They play a crucial role in the regulation of the reproductive axis. KP is a stimulator of the reproductive axis, while GnIH is the inhibitory opponent (Pinilla *et al*. 2012, Ubuka *et al*. 2013). Diverse data from a wide variety of vertebrate taxa indicate that these neuropeptides play a role in the regulation of reproduction in response to short-term (circadian) and long-term (seasonal) environmental changes (Pinilla *et al*. 2012, Clarke & Parkington 2014). Moreover, both neuropeptides act as conduits/mediators for transferring the impact of body internal cues such as gonadal steroids and current energy status to the reproductive axis (Smith *et al*. 2007, Clarke *et al*. 2012, Smith 2013).

GnIH, which is also known as RF amide-related peptide 3 (RFRP3) in mammals, is an orexigenic neuropeptide (Tachibana *et al*. 2005, McConn *et al*. 2014). However, the involvement of GnIH in the metabolic regulation of the hypothalamic–pituitary–gonadal (HPG) axis is not clearly known, but increasing data propose an involvement of this neuropeptide in negative energy balance-induced alterations in the HPG axis in mammals, including primates (Batool *et al*. 2014, Sabet Sarvestani *et al*. 2014). Recently, GnIH receptor (GnIHR) antagonist, RF9, injection has markedly reversed fasting-induced quiescent of the reproductive axis in the male adult rhesus monkey (Batool *et al*. 2014). In contrast, an anorexigenic effect of KP has been noted (Stengel *et al*. 2011). More importantly, KP has been advocated as a major signal for transferring body metabolic status-related information to the neuroendocrine reproductive axis (Castellano *et al*. 2010*a*,*b*, Sanchez-Garrido & Tena-Sempere 2013, Wahab *et al*. 2013*a*).

The hypothalamic KP neurons have been reported to express receptors for leptin (Smith *et al*. 2006). In addition, the *Kiss1* transcript level in the hypothalamus is at its lowest level in conditions of hypoleptinemia (Smith *et al*. 2006, Luque *et al*. 2007). Moreover, leptin injection markedly heightens *Kiss1* expression in the hypoleptinemic condition (Smith *et al*. 2006, Luque *et al*. 2007). A brief period of food restriction has been noted to alter *Kiss1* as well as KP receptor (Kiss1r) expression (Castellano *et al*. 2005, Wahab *et al*. 2011*a*).

Injection of exogenous KP reverses brief food restriction-induced reproductive quiescence in rats and monkeys (Castellano *et al*. 2005, Wahab *et al*. 2008, 2014). Although KP significantly increased brief food restriction-induced attenuation of testosterone release, the response of the reproductive axis to KP administration, both in commencement and magnitude, is notably affected in adult male rhesus monkeys (Wahab *et al*. 2008, 2014). These observations support a possible involvement of GnIH and KP in the mediation of metabolic influence on the reproductive axis performance.

In this article, we will summarize the presently available data obtained from experimental animals, for both nonprimates and primates, implicating GnIH–GnIHR and KP-Kiss1r signaling as potential vital pathways, which modify reproductive activities according to the body's existing metabolic reserves.

Role of GnIH and KP in regulation of reproduction

Role of GnIH in regulation of reproduction

About 14 years ago, GnIH was first identified in the quail brain as an inhibitor of the reproductive axis (Tsutsui *et al*. 2000). Later on, its mammalian orthologs were discovered to be encoded by *RFRP* (*NPVF*) gene (Hinuma *et al*. 2000, Yoshida *et al*. 2003). Now, globally, RFRP3 is regarded as the mammalian GnIH ortholog (Ubuka *et al*. 2013, Osugi *et al*. 2014). In this article, we will uniformly use GnIH for both avian and mammalian peptides.

GnIH neurons are mainly concentrated in the dorsomedial hypothalamus. The axonal fibers of these neurons form close appositions with GnRH-containing neurons in several species including nonhuman primates and humans (Kriegsfeld *et al*. 2006, Johnson *et al*. 2007, Smith *et al*. 2008, Ubuka *et al*. 2008, 2009*a*). This anatomical evidence suggests that GnIH can directly alter intermittent GnRH discharge from the hypothalamus. Indeed, it has been documented that direct GnIH application to GnRH neurons reduces the firing rate of a subpopulation of these neurons (Ducret *et al*. 2009). Besides this action on GnRH neurons, GnIH was shown to cross-talk with the HPG axis at the level of anterior pituitary gonadotropes, which express GnIHR. Moreover, GnIH may also act directly at the gonadal level (Ubuka *et al*. 2009*b*, 2014, Sari *et al*. 2009, McGuire & Bentley 2010, Anjum *et al*. 2014).

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GnIH exerts actions through its putative G-protein-coupled receptors (GPR), e.g., GPR147. GnIHR is expressed in several brain areas including GnRH-containing neurons (Fig. 1; Ubuka *et al*. 2008, 2009*b*, 2012, Rizwan *et al*. 2012). In addition to its hypothalamic expression, GnIHR expression has been reported in the pituitary (Ubuka *et al*. 2009*b*) and the gonads (McGuire & Bentley 2010). The inhibitory effect of GnIH on reproductive hormones secretion can be blocked by the GnIHR antagonist RF9 (Pineda *et al*. 2010*a*,*b*, Rizwan *et al*. 2012).

Several functions, which relate to the regulation of reproduction and behavior, have been assigned to GnIH–GnIHR signaling on the basis of unveiling evidence over the years. This ligand-receptor duo has been described in negative feedback from gonadal steroids, as well as metabolic, circadian, and seasonal regulation of reproduction (for a review, see Smith & Clarke (2010), Clarke (2011), Smith (2012) and Clarke & Parkington (2014)). While reviewing all of the data on the role of GnIH in the regulation of reproduction is beyond the scope of this review, here we will describe evidence suggesting its involvement in the metabolic regulation of reproduction.

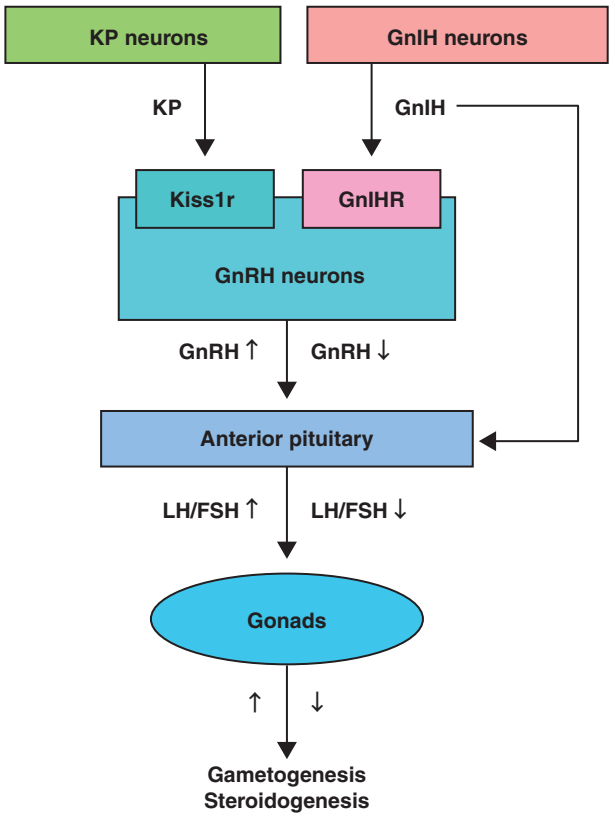

Figure 1
GnIH and KP signaling in the regulation of reproduction. The hypothalamic KP expressing neurons provide a tonic stimulatory drive to the neuroendocrine reproductive axis while GnIH neurons generate an inhibitory drive to the reproductive axis. KP acts via its G protein-coupled receptor, *Kiss1r*, while GnIH exerts actions via its G protein-coupled receptor, *GnIHR*. Expressions of both *Kiss1r* and *GnIHR* have been reported on the hypothalamic GnRH-containing neurons. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-14-0688.

Role of KP in regulation of reproduction

On the basis of a large number of molecular and physiological evidence, hypothalamic KP Kiss1r signaling has been implicated in the central control of the reproductive axis in a number of vertebrate species (Fig. 1; reviewed in Clarke (2011), Hameed *et al*. (2011), Wahab *et al*. (2011*b*), George & Seminara (2012), Pinilla *et al*. (2012) and Terasawa *et al*. (2013)). The finding of the involvement of KP and its receptor *Kiss1r* in the initiation and maintenance of reproduction is considered as one of the most important discoveries made in the field of reproductive neuroendocrinology (Seminara & Kaiser 2005). Since the discovery of GnRH in the late 1970s, many new peptides/neurotransmitters have been described in the neuroendocrine control of reproduction, but none has had such a dramatic effect as KP (Seminara & Kaiser 2005, Pinilla *et al*. 2012).

In 2003, two groups of researchers discovered, independently from each other, that loss of function mutations of *KISS1R* caused hypogonadotropic hypogonadism in humans and were associated with the delay or absence of sexual maturation (De Roux *et al*. 2003, Seminara *et al*. 2003). Targeted deletions of *Kiss1r*/*Kiss1* result in a lack of sexual behavior and a low level of gonadotropin in mice (Funes *et al*. 2003, Seminara *et al*. 2003, D' Anglemont de Tassigny *et al*. 2007, Lapatto *et al*. 2007). In addition, an activating mutation has also been described in *KISS1R* in humans, which leads to an early attainment of reproductive ability in pubertal females, because this mutation (Arg386Pro) results in a long-lasting activation of KISS1R in response to KP (Teles *et al*. 2008). Therefore, an intact KP-KISS1R system is considered to be very important in order to achieve reproductive capacity and its continuation in adults (reviewed in Seminara & Kaiser (2005), Hameed *et al*. (2011), Wahab *et al*. (2011*b*), Pinilla *et al*. (2012) and Terasawa *et al*. (2013)).

Exogenous KP injection either centrally or peripherally causes stimulation of the HPG axis in many mammalian species (Thompson *et al*. 2004, Shahab *et al*. 2005, Lents *et al*. 2008, Pinilla *et al*. 2012, Okamura *et al*. 2013). Central as well as peripheral administration of KP10, the shortest ten amino acids peptide hormone in the KP hormone group,

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or KP54, the longest one, robustly increases systemic levels of the luteinizing hormone (LH) and the follicle-stimulating hormone in sexually immature and mature rodents (Gottsch *et al.* 2004, Navarro *et al.* 2004, 2005*a*,*b*, Thompson *et al.* 2004). I.c.v. or peripheral KP10 administration has been shown to significantly augment GnRH-dependent LH secretion in gonadectomized sexually immature male monkeys (Shahab *et al.* 2005). In addition, KP10 administration increases systemic testosterone levels in adult male rats (Thompson *et al.* 2004) and monkeys (Wahab *et al.* 2008). The stimulatory effects of KP to hormones on gonadotropins are blocked by GnRH antagonists, indicating that the effect of KP on the reproductive axis is dependent on GnRH receptor (GNRHR) signaling and not directly through *Kiss1r* in the pituitary (Gottsch *et al.* 2004, Irwig *et al.* 2004, Matsui *et al.* 2004, Shahab *et al.* 2005). Moreover, KP10 administration in sheep causes an increase in the concentration of GnRH in cerebrospinal fluid (Messager *et al.* 2005), confirming that the action of KP on the HPG axis is mediated via modulation of GnRH release.

Therefore, the KP-induced stimulation of the HPG axis occurs via the hypothalamic GnRH neuronal network. Evidence exists for both direct and indirect KP signals to GnRH neuronal networks. The following evidence indicates that KP communicates directly with hypothalamic GnRH-secreting cells. Firstly, most GnRH-secreting cells express *Kiss1r* (Irwig *et al.* 2004, Parhar *et al.* 2004, Han *et al.* 2005, Messager *et al.* 2005). Secondly, KP-immunoreactive fibers are found in close apposition with GnRH-secreting cells (Clarkson & Herbison 2006, Decourt *et al.* 2008, Smith *et al.* 2008). Thirdly, KP can act *per se* on *in vitro* GnRH-secreting cells to heighten their firing rates (Quaynor *et al.* 2007, Dumalska *et al.* 2008, Liu & Herbison 2008, Liu *et al.* 2008, Pielecka-Fortuna *et al.* 2008). Fourthly, there has been recent evidence suggest that KP can communicate indirectly with GnRH neurons through other neurons such as glutamatergic and GABAergic neurons (Pielecka-Fortuna *et al.* 2008, García-Galiano *et al.* 2012, Di Giorgio *et al.* 2013).

Compelling data from various vertebrates, including mammals, suggest that KP is also involved in the mediation of gonadal steroid-negative feedback, estradiol-positive feedback during ovulation, as well as seasonal, circadian, metabolic, and stress signals to the reproductive axis (Smith 2009, 2013, Castellano *et al.* 2010*a*,*b*, Clarke & Caraty 2013, Kriegsfeld 2013, Sanchez-Garrido & Tena-Sempere 2013, Wahab *et al.* 2013*a*). In this article, we reviewed KP involvement in the metabolic regulation of reproduction.

Evidence demonstrating the involvement of GnIH and KP in the metabolic regulation of reproduction

Presence of GnIH and KP containing cells in the brain center for feeding and reproduction control

Besides many other functions, the hypothalamus is the brain center for the regulation of feeding and reproduction (Kennedy & Mitra 1963*b*, Wynne *et al.* 2005). It serves as the central regulator of neuroendocrine function by controlling pituitary hormone secretion directly or indirectly. It contains a number of neuropeptides such as agouti-related peptide (AgRP), melanin-concentrating hormone, galanin-like peptide, orexin, 26RFamide, ghrelin, neuropeptide-Y (NPY), proopiomelanocortin (POMC)-derived peptides, and cocaine- and amphetamine-regulated transcript (CART), which play important roles in the regulation of feeding (Kageyama *et al.* 2005, Wynne *et al.* 2005, Crown *et al.* 2007, Hill *et al.* 2008, Wu *et al.* 2009, Galusca *et al.* 2012). Some of these are orexigenic neuropeptides while others are anorexiegenic neuropeptides.

KISS1 and *GnIH* mRNA expressing neurons have also been consistently reported in hypothalamic tissues of diverse taxa including primates (Irwig *et al.* 2004, Castellano *et al.* 2005, Shahab *et al.* 2005, Smith *et al.* 2008, Ubuka *et al.* 2009*a*,*b*, Escobar *et al.* 2013, Sabet Sarvestani *et al.* 2014, Ohga *et al.* 2015). As discussed previously, the peptide products of these two genes have been implicated as the main central regulator of the reproductive axis (reviewed in Smith & Clarke (2010), Tsutsui *et al.* (2010), Clarke (2011), Pinilla *et al.* (2012), Tena-Sempere *et al.* (2012) and Ogawa & Parhar (2014)). Moreover, these neuropeptides also modulate food intake. GnIH has been noted to stimulate feeding (Clarke *et al.* 2012, McConn *et al.* 2014) while KP has an anorexigenic effect in fasted mice (Stengel *et al.* 2011). Significantly, early studies in rats did not observe any change in food intake after KP injection under both food restriction and *ad libitum* feeding conditions (Thompson *et al.* 2004, Castellano *et al.* 2005). This difference in findings may be due to species differences or variations in experimental set up. Automated noninterfering food intake monitoring was performed in studies with mice, while in studies with rats a manual assessment was done (Thompson *et al.* 2004, Castellano *et al.* 2005, Stengel *et al.* 2011).

These neurons, containing KP and GnIH, are also expressing receptors of the metabolic hormones (Smith *et al.* 2006, Poling *et al.* 2014, Ratra & Elias 2014). Importantly, the activities of these neurons, as will be

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discussed below, are altered during negative energy balance conditions (Castellano & Tena-Sempere 2013, Wahab *et al*. 2013*a*, Roa & Tena-Sempere 2014). Also, these neurons have been documented to directly cross-talk with GnRH-containing neurons (Irwig *et al*. 2004, Parhar *et al*. 2004, Han *et al*. 2005, Messager *et al*. 2005, Clarkson & Herbison 2006, Decourt *et al*. 2008, Smith *et al*. 2008, Ubuka *et al*. 2008, 2009*b*, 2012, Rizwan *et al*. 2012). Moreover, expression of KP and GnIHRs has been noted on GnRH neurons (Irwig *et al*. 2004, Parhar *et al*. 2004, Han *et al*. 2005, Messager *et al*. 2005, Ubuka *et al*. 2009*b*). Therefore, this multifaceted neural circuit of hypothalamic neurons can act to integrate alterations in metabolic homeostasis with the neuroendocrine regulation of the HPG axis function.

GnIH and KP can also indirectly convey the body's current energy reserve-related information to the neuroendocrine center controlling reproductive axis. Several studies have demonstrated GnIH and KP receptor expression (Shahab *et al*. 2005, Clarkson & Herbison 2006, Kriegsfeld *et al*. 2006, Decourt *et al*. 2008, Ubuka *et al*. 2008) in many different areas of the hypothalamus. Backholer *et al*. (2010) reported the existence of reciprocal input between the hypothalamic KP-containing neurons and anorexigenic POMC neurons and orexigenic NPY neurons in the ovine hypothalamus, while Qi *et al*. (2009) documented that GnIH neurons project to NPY, POMC, and orexin-containing neurons in sheep brains. These anatomical connections propose an essential role of GnIH and KP cells in integrating current metabolic status to the HPG axis output. Indeed, GnIH and KP administrations altered expression of both *POMC* and *NPY* mRNA (Fu & van den Pol 2010, Kim *et al*. 2010, Jacobi *et al*. 2013). In addition, KP directly stimulates POMC cells, while it indirectly suppresses hypothalamic NPY cells (Fu & van den Pol 2010). Jacobi *et al*. (2013) reported a negative effect of GnIH on POMC cells (Jacobi *et al*. 2013). They also noted a negative effect of GnIH on the activity of NPY cells in mice. Of note, it has been shown that activation of the NPY-Y1 receptor is required for the physiological amplification of the spontaneous preovulatory LH surge in rats (Leupen *et al*. 1997). Therefore, GnIH inhibition of NPY cells can also contribute to the inhibitory effect of GnIH on LH surge.

The above mentioned data indicate both a direct and indirect involvement of GnIH and KP in linking metabolic status-related information with the feeding or reproductive axis (Fig. 2). Reciprocal input from orexigenic and anorexigenic neuronal systems to GnIH and KP-containing neurons is also possible, which may affect

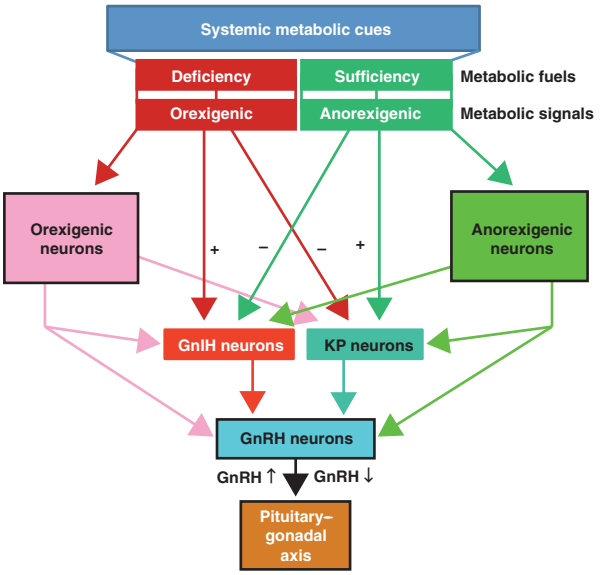

Figure 2
Schematic representation of the interaction of metabolic cues with GnIH and KP signaling. Metabolic fuel deficiency and sufficiency differentially modulate the activities of KP and GnIH containing neurons. The deficiency of metabolic cues, either directly or indirectly via orexigenic factors, decreases KP neuronal activities and increases GnIH neuronal activities. In contrast, metabolic cue sufficiency, either *per se* or through anorexigenic signals, decreases GnIH neuron activities and increases the activities of KP neurons. GnIH and KP neurons can receive current metabolic status-related information from orexigenic and anorexigenic neurons as well. Likewise, orexigenic and anorexigenic neurons can also directly convey current metabolic status-related information to GnRH neurons. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-14-0688.

RFRP and *KISS1* expression as well as the release of their peptide products. In support of this notion, Luque *et al*. (2007) noted a defective expression of *Kiss1* in *Npy*-knockout mice and suggested that a normal NPY neuronal network is essential for KP-containing neurons to function accurately. Similarly, dynorpin A and neurokinin B coexpressions have been identified on KPergic neurons (Goodman *et al*. 2013). It will be interesting to know whether KP and GnIH neurons contain receptors for *AgRP*, *NPY*, *GALP*, *CART*, and *POMC* or receive afferent projections from these neuronal networks of the feeding and satiety center. Of note, reciprocal connections from *POMC* and *NPY* to KP neurons in the ovine brain have already been shown by Backholer *et al*. (2010). The functional significance of this connection is supported by a positive effect of melanocortin agonist on KP neurons in the preoptic area of ewes (Backholer *el al*. 2009). In the same vein, a potent stimulatory effect of CART, another anorexigenic neuropeptide of the hypothalamus, has also been noted on KP neurons (True *et al*. 2013). Recently, electrophysiological studies have demonstrated the

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importance of KP for the stimulatory effect of NPY antagonism on the GnRH (Verma *et al*. 2014). Although there are no *in vivo* data on the effect of NPY on KP neuronal activities, an *in vitro* study reported a stimulatory effect of NPY on *Kiss1* expression in hypothalamic cell line N6 (Luque *et al*. 2007).

KP and GnIH signaling during puberty

Puberty is an important stage in mammalian reproduction. It is characterized by prominent changes in the activity of the reproductive axis (Terasawa & Fernandez 2001). The starting mechanisms of puberty have begun to be properly deciphered in light of recent advances in neuroendocrinology of reproduction. Puberty is regulated by both genetic and nongenetic factors. The important role of metabolic cues in puberty regulation is well established. Many studies have reported that an altered energy balance at the time of puberty severely affects the normal activation of the reproductive axis (Wade & Jones 2004, Castellano *et al*. 2010*a*).

During the prepubertal period, the body's metabolic reserves exert a vital influence on the pubertal awakening of the HPG axis (Kennedy & Mitra 1963*a*, Foster & Olster 1985). Metabolic stress and energy imbalance conditions in humans, such as anorexia, morbid obesity, are characterized by perturbation in the achievement of reproductive capacity at the time of puberty (Castellano *et al*. 2010*a*, Sanchez-Garrido & Tena-Sempere 2013). A large number of studies have demonstrated that metabolic insufficiency during this critical period of reproductive ability realization delays or prevents the awakening of the HPG axis in rats (Kennedy & Mitra 1963*a*, Schenck *et al*. 1980), mice (Marsteller & Lynch 1983, Perrigo & Bronson 1983), lambs (Foster & Olster 1985), Syrian hamsters (Morin 1975), and humans (Teles *et al*. 2008).

Metabolic imbalance has an impact on the reproductive axis via alteration in KP signaling (Wahab *el al*. 2013*a*,*b*). In nonprimate studies, food restriction delayed the achievement of puberty hallmarks, compared with the control condition, while KP injections caused early achievement of puberty (Castellano *et al*. 2005). These data indicate that metabolic insufficiency attenuated KP release during this critical period. Indeed, *Kiss1* expression is reduced under food restriction conditions (Castellano *et al*. 2005, Wahab *et al*. 2011*a*, Sabet Sarvestani *et al*. 2014). In mammalian studies, a permissive role of the metabolic hormone leptin has been suggested in puberty and reproduction (Grumbach 2002, Terasawa *et al*. 2012, Sanchez-Garrido & Tena-Sempere 2013). However, it is unclear whether leptin acts at the hypothalamic, pituitary, or gonadal level to permit activation of the reproductive axis. Available data indicate all these possibilities. *LepR* is expressed on many different hypothalamic neuronal populations, including KP neurons that directly or indirectly modulate GnRH neuronal activity (Quennell *et al*. 2009, Donato *et al*. 2011, Zuure *et al*. 2013, Bellefontaine *et al*. 2014, Martin *et al*. 2014). The action of leptin on pituitary gonadotropes is suggested by the fact that these cells express the LepR, and leptin modulates the LH surge (Crane *et al*. 2007, Akhter *et al*. 2014). A more recent study has shown that loss of LepR in gonadotropes results in reduced GNRHR expression and LH secretion (Akhter *et al*. 2014).

However, there are no data on the impact of changes in metabolic cues on the GnIH neuronal activity during prepuberty or at the onset of puberty. Therefore, it will be worthwhile for future studies to focus on the interaction between metabolic cues and GnIH in prepubertal and pubertal stages.

KP and GnIH signaling in metabolic insufficiency conditions of fasting

The performance of the reproductive axis during various time periods of food restriction has been extensively studied in mammals (Marsteller & Lynch 1983, Cameron 1996, Shahab *et al*. 1997, Wahab *et al*. 2008, 2014). Both short- and long-term food restrictions have been observed to cause quiescence of the HPG axis. In macaques, it has been noted that merely missing a single daily meal or a change in its timing can cause reproductive quiescence (Cameron 1996). Besides modulation of pituitary GNRHR expression (Crane *et al*. 2007), food restriction also induces reproductive quiescence by a decrease in GnRH release from the hypothalamus (Bergendahl *et al*. 1991, Aloi *et al*. 1997, Wahab *et al*. 2008). Nevertheless, the mechanism by which food restriction disturbs neural circuits that govern the pulsatile GnRH release is not fully understood at present.

Recently, we have carried out an analysis of the KP effect on the reproductive quiescence in various time periods of fasting in adult male rhesus macaques. I.v. injections of KP10, a decapeptide of the KP hormone family, caused a prominent augmentation of plasma testosterone levels in 12, 18, 24, and 48 h food-restricted monkeys (Wahab *et al*. 2008, 2014). In terms of initiation and magnitude, KP injection-induced testosterone response in the 12-h fasted macaque monkey was similar to that of normally fed monkeys. However, the KP10 injection-induced testosterone response was lowered both

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in initiation and magnitude in 18, 24, and 48-h food-deprived monkeys (Wahab *et al*. 2008, 2014). Therefore, these observations suggest that injection of exogenous KP incompletely reverses more than 12-h food restriction-induced reproductive quiescence. These results suggest that brief fasting conditions may cause a drop in KPergic neuron output to the neuronal network regulating the pulsatile release of GnRH, which in turn causes a decline in LH and testosterone systemic levels. Recently, we have reported in another study a reduction in *Kiss1* mRNA in the hypothalami of food-deprived macaques (Wahab *et al*. 2011*a*).

A number of studies in other models have also demonstrated a marked attenuation in *Kiss1* transcript levels in the hypothalamus under food restriction conditions along with inhibition of the reproductive axis. Injections of exogenous KP reverse the reproductive quiescence in these animals (Castellano *et al*. 2005, Luque *et al*. 2007). On the basis of available data, it is well established that the hypothalamus is the major site of KP action on the reproductive axis (Batool *et al*. 2014). Therefore, in a fasting situation, KP-induced stimulation of the reproductive axis also occurs via its action on the hypothalamic GnRH release. Nevertheless, whether KP acts directly or indirectly is still not clear. Based on observations, a direct action is suggested by the evidence that GnRH neurons express *Kiss1r* (Irwig *et al*. 2004, Parhar *et al*. 2004, Han *et al*. 2005, Messager *et al*. 2005), while in an indirect action KP modulates the activity of other hypothalamic neuronal networks which crosstalk with GnRH neurons (Fu & van den Pol 2010, Kim *et al*. 2010).

Recently, we have reported that GnIHR antagonism via injections of RF-9 in food-deprived macaques reinstated lowered levels of testosterone to the normal levels of fed animals (Batool *et al*. 2014). From this, one can infer that GnIH is one of the main inhibitory drivers to the reproductive axis in negative energy balance conditions, while the blocking of GnIH action permits reproduction. However, it is still unclear where GnIH crosstalk takes place with the reproductive axis. Whether GnIH prevents testosterone release in fasted animals indirectly via constraining GnRH pulsatile release and/or preventing LH secretion from pituitary gonadotrophs, or directly by acting *per se* on testes to stop testosterone release, warrants further scholarly investigation.

The nature of the fasting metabolic insufficiency-induced signals that alter GnIH and KP signaling is not known. However, a possible mechanism through which fasting may alter GnIH and KP signaling is illustrated in Fig. 2. Important candidates in this respect are peripheral metabolic cues and their receptive hypothalamic neuronal circuits. Important metabolic cues are adiponectin, glucose, ghrelin, cortisol, insulin, leptin, or other metabolic molecules (Kinoshita *et al*. 2003, Budak *et al*. 2006, Wahab *et al*. 2010, 2011*c*, 2013*a*, Poling *et al*. 2014, Roa & Tena-Sempere 2014). Glucose is a ubiquitous cue for all organisms. It is the primary fuel for neurons. Therefore, it can mediate metabolic insufficiency-related information to GnIH and KP neurons. But this notion is ruled out by findings that in acute insulin-induced hypoglycemia, the sensitivity of GnRH cells to KP treatment remains preserved (Wahab *et al*. 2012).

Leptin, ghrelin, and cortisol are other important candidates. All of these are implicated in altering the expression of hypothalamic *Kiss1* (Smith *et al*. 2006, Luque *et al*. 2007, Forbes *et al*. 2009, Kinsey-Jones *et al*. 2009). Important roles of the adipokine hormone leptin in the metabolism and reproduction have recently been extensively reviewed (e.g., Moschos *et al*. 2002, Budak *et al*. 2006, Sanchez-Garrido & Tena-Sempere 2013, Tena-Sempere 2013, Nestor *et al*. 2014, Roa & Tena-Sempere 2014). The proper functioning of various hypothalamic neuronal populations is sensitive to systemic leptin levels (Casanueva & Dieguez 1999, Pralong & Gaillard 2001, Moschos *et al*. 2002). Recently, KP neurons have been noted to express LepR, the receptor of leptin. In mice, about 40% of KPergic neurons in the arcuate nucleus express *LepR*s (Smith *et al*. 2006). In sheep, expression of LepR has also been documented on KP-containing neurons in the medial preoptic area (Backholer *et al*. 2010). In situations of altered energy balance including food restriction, low systemic concentration of leptin leads to a low hypothalamic level of *Kiss1* mRNA (Castellano *et al*. 2005, Smith *et al*. 2006, Luque *et al*. 2007, Wahab *et al*. 2011*a*), while administration of leptin greatly ameliorates transcript levels of *Kiss1* (Smith *et al*. 2006, Luque *et al*. 2007, Backholer *et al*. 2010). In lean sheep, a model of energy deficiency characterized by low stores of energy reserves, hypogonadotropic hypogonadism and low leptin levels, reduced *Kiss1* mRNA expression has been observed in both the arcuate nucleus (ARC) and preoptic area (Backholer *et al*. 2010). Consequently, leptin infusion increases *Kiss1* mRNA expression in both the ARC and preoptic area in these animals. This finding suggests a role of both the ARC and preoptic area in relaying metabolic information to the reproductive axis. Altogether, these observations propose that leptin is one of direct metabolic signals that modulate the functioning of KP-containing neurons in the hypothalamus during situations of negative energy balance and hence, ultimately, reproductive output. Nevertheless, recent

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studies have demonstrated that leptin transfers information about metabolic status to KP-containing neurons indirectly because leptin administration fails to phosphorylate the STAT3, a key component of a major intracellular signaling pathway mediating leptin action, in KP neurons (Louis *et al.* 2011). Indeed, leptin receptors are located on many neuronal populations in the hypothalamus (Wada *et al.* 2014) which cross-talk with KPergic neurons (Backholer *et al.* 2010, Fu & van den Pol 2010, Kim *et al.* 2010).

Ghrelin, a gut metabolic hormone, stimulates food intake (Pusztai *et al.* 2008). Recently, it has been shown to reduce *Kiss1* transcript levels in the hypothalamus. Exogenous injections as well as food restriction induced hyperghrelinemia have been documented to decrease mRNA levels of *Kiss1* (Forbes *et al.* 2009, Frazao *et al.* 2014). These findings indicate that ghrelin-induced reduction in hypothalamic *Kiss1* expression can be one of the key factors in metabolic deficiency-induced inhibition of the reproductive axis. At this time, the effect of ghrelin on the activity of GnIH-containing neurons is not known.

Although recently it has been noted that GnIH neurons are not responsive to leptin, glucocorticoid receptor expression has been observed on GnIH-containing neurons which are upregulated by both acute and chronic stress (Gojska & Belsham 2014, Poling *et al.* 2014, Rizwan *et al.* 2014). As fasting is a type of metabolic stress and an increase in glucocorticoid has been reported in this condition (Wahab *et al.* 2008), it is possible that glucocorticoid may alter the GnIH system, which may communicate inhibitory information to the reproductive axis (Smith & Grove 2002). Moreover, the KP neuronal system is also negatively affected by stress signals (Kinsey-Jones *et al.* 2009). Whether GnIH and KP neuronal systems are also modulated by changes in glucocorticoids during metabolic stress warrants further investigation. More importantly, it would be worthwhile to study the level of impact of stress vs metabolic signals on KP and GnIH systems during metabolic insufficiency conditions.

KP and GnIH signaling in conditions of altered energy balance and reproduction

In contrast to metabolic insufficiency situations of fasting, there are some conditions of altered energy balances such as diabetes and obesity, etc. in which more than enough stores of metabolic energy are present, but the body cannot use them (Sexton & Jarow 1997, Cleary *et al.* 2001, Smith *et al.* 2010*a*). Therefore, in these conditions, the malfunction of the reproductive axis has also been attributed to an altered energy balance. In this part, we summarize the data that proposes the role of GnIH and KP in modifying the HPG axis in various conditions of energy imbalance (Table 1).

Lactation During lactation, the negative energy balance and suckling stimulus have been implicated in the suppression of the reproductive axis (Russell *et al.* 2001, Smith & Grove 2002). The negative energy balance results from a huge energy drain due to the production of milk. The lactation-induced reproductive quiescence is caused by impairments at both upstream and downstream of GnRH (Smith 1978, Lee *et al.* 1989). Reduced levels of GNRHR in the pituitary and impaired pituitary responsiveness to GnRH have been suggested as possible downstream mechanisms (Smith 1978), while alterations in hypothalamic pathways involving KP and GnIH have been suggested as an upstream mechanism.

Hypothalamic GnIH–GnIHR signaling and KP-Kiss1r signaling are potential pathways that can relay negative energy balance and suckling stimulus-related information to the GnRH neuronal network as shown in Fig. 3 (Smith *et al.* 2010*a*, Liu *et al.* 2014). An obvious increase in GnIH was reported in the hypothalamus of lactating

Table 1 Summary of *Rfrp* and *Kiss1* mRNA expression in various paradigms of altered metabolic homeostasis

Metabolic paradigm	*Kiss1* mRNA	*RFRP* mRNA	Reference
Fasting	−	+	Castellano *et al.* (2005), Wahab *et al.* (2011*a*) and Jahanara *et al.* (2014)
Lactation	−	+	Yousefabad *et al.* (2013) and Liu *et al.* (2014)
STZ-induced diabetes	−	?	Castellano *et al.* (2006)
Diet-induced obesity	−,=	=	Quennell *et al.* (2011), Luque *et al.* (2007) and Rizwan *et al.* (2014)
Ob/Ob mice	−	=	Smith *et al.* (2006), Quennell *et al.* (2011) and Rizwan *et al.* (2014)

+, increase; −, decrease; =, no effect; ?, currently unknown.

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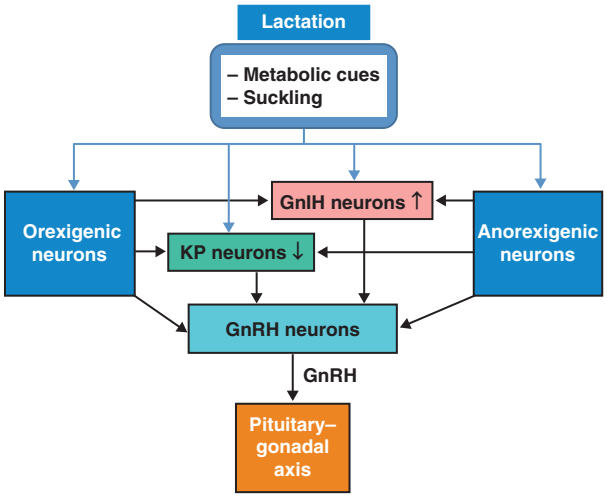

Figure 3
A possible mechanism of lactation-induced suppression of the reproductive axis. Metabolic cues and suckling stimulus during lactation can alter the activities of KP and GnIH-containing cells both directly and indirectly. In the former case, lactation-specific metabolic changes increase the activities of GnIH neurons and attenuate KP neuronal activities. These neurons then act accordingly on the GnRH neurons. In the latter case, KP and GnIH neurons receive information from orexigenic and anorexigenic neurons and, in turn, convey the impact of this information to the reproductive axis via GnRH neurons. Likewise, orexigenic and anorexigenic neurons can also convey lactation-specific information to GnRH neurons. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-14-0688.

gonadectomized rats, while *Kiss1* transcript levels were markedly attenuated (Yamada *et al.* 2007, Yousefabad *et al.* 2013, Liu *et al.* 2014). Moreover, these gonadectomized rats maintain normal responsiveness to injection of KP. KP administration-induced LH response was comparable between lactating and nonlactating female rats (Yamada *et al.* 2007). Although Roa *et al.* (2006) noted that the magnitude of KP-induced LH secretory responses was greatly reduced in lactating female rats as compared with pair-control animals. Thus, a noteworthy influence of lactation on the GnRH neuronal sensitivity to KP in terms of gonadotropin secretion was reported. Altogether, these results suggest that an increase in expression of the *Rfrp* gene during lactation may cause higher synthesis and release of GnIH. In addition, a decrease in the *Kiss1* gene expression may lead to lower KP synthesis and release. These alterations in GnIH and KP could reduce the intermittent discharge of GnRH and thus ultimately lead to inhibition of the HPG axis during lactation. Presently, the nature of the factors responsible for the stimulation of *Rfrp* and attenuation of *Kiss1* expression during lactation is unclear, but it is logical to predict them as a combination of different peripheral and central signals that cause a rise in lactation-associated food ingestion. It has been reported that circulating leptin levels are lowered in lactating rats compared with nonlactating animals (Denis *et al.* 2003). Moreover, lactation is associated with reduced expression of anorexigenic POMC and increased expression of orexigenic AGRP and NPY (Suzuki *et al.* 2014). Since leptin, POMC, AGRP, and NPY affect the KP/GnIH–GnRH system, they can also modulate *Rfrp* and *Kiss1* expression during lactation. Of note, a very recent study demonstrated an important role of prolactin in the lactation-induced suppression of KP release and reproduction (Brown *et al.* 2014).

Obesity A good model for studying the pathophysiology of obesity is the *Ob/Ob* mice, which is deficient in the leptin encoding gene (Mounzih *et al.* 1997, Cleary *et al.* 2001). These mice develop severe obesity along with a malfunctioning reproductive system. The main cause of hypogonadism in these mice is considered to be of central hypothalamic origin because the gonadal response to LH and pituitary sensitivity to GnRH remain preserved (Mounzih *et al.* 1997, Cleary *et al.* 2001). Of note, it was noted that these mice have disrupted expression of *Kiss1* mRNA in the hypothalamus (Smith *et al.* 2006). Exogenous applications of leptin have been noted to augment *Kiss1* gene expression in this model of obesity (Smith *et al.* 2006). As a number of studies have reported that leptin infusions also rescue reproductive quiescence in *Ob/Ob* mice (Mounzih *et al.* 1997, Cleary *et al.* 2001), we cautiously propose that the impaired KP-Kiss1r signaling contributes at least partly to reproductive impairments caused by leptin deficiency.

More recently, analyses of the function of leptin on the GnIH–GnIHR system have been carried out in these mice (Poling *et al.* 2014, Rizwan *et al.* 2014). Only a small subset of GnIH neurons expresses the *LepR* (Poling *et al.* 2014). These results suggest a lack of an essential role of the GnIH in linking leptin with reproductive function under these conditions. Rizwan *et al.* (2014) did not observe any differences in hypothalamic *Rfrp* gene mRNA levels or GnIH-immunoreactive cell number in *Ob/Ob* and control animals, or in high-fat and low-fat diet-fed control mice. In addition, they did not detect expression of *LepR* in GnIH cells. Moreover, they did not see leptin-induced STAT3 in vicinity to GnIH-containing neurons, but there was a small amount (2–13%) of co-localization and no noteworthy differences between control and leptin-injected mice (Rizwan *et al.* 2014).

Diabetes Diabetes, a metabolic disorder, is characterized by either dysfunction of pancreatic β-cells or resistance to insulin, an important metabolic hormone. Hyperglycemia

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is a hallmark of diabetes (Spindler-Vomachka & Johnson 1985, Sexton & Jarow 1997, Clough *et al*. 1998). Diabetic patients as well as animal models of diabetes are characterized by secondary hypogonadism. A number of studies have reported preserved GnRH content as well as its secretory capacity in the hypothalamus, suggesting that the central cause of reproductive impairment in diabetic human patients and experimental diabetic animals lies upstream of GnRH neurons (Howland & Zebrowski 1976, 1980, Spindler-Vomachka & Johnson 1985, Sexton & Jarow 1997, Clough *et al*. 1998). This notion is supported by recent studies in human diabetic patients and diabetic rats, which demonstrated that pituitary response in terms of LH discharge to exogenous KP injections remains conserved (Castellano *et al*. 2006, George *et al*. 2013). Therefore, the primary defect responsible for quiescence of the reproductive axis more likely involves a reduction in KP stimulatory drive to the hypothalamic GnRH cells. Of note, hypothalamic *Kiss1* expression is greatly decreased in diabetic rats (Castellano *et al*. 2006).

In the diabetic model, hypoleptinemia has been suggested to more likely be a causative agent responsible for reduced *Kiss1* expression because infusion of exogenous leptin markedly enhanced hypothalamic levels of *Kiss1* mRNA, while insulin-like growth factor and insulin failed to do so (Luque *et al*. 2007). Although Luque *et al*. (2007) did not observe an effect of insulin on *Kiss1* expression, the presence of an insulin receptor (IR) on KP neurons in the ARC of the hypothalamus gives a clue as to the importance of insulin-KP signaling. Indeed, a recent study by Qiu *et al*. (2013) has demonstrated that specific ablation of the IR from KP neurons results in delayed puberty although no impact on the reproductive capacity of adult animals is noted.

Pregnancy Pregnancy is a physiological condition that is characterized by a rise in the body's basal metabolic rate along with a decrease in hypothalamic GnRH and pituitary LH discharge (Linkie & Niswender 1972, Morishige *et al*. 1973, Boyle & Roth 2012). Recently, Sabet Sarvestani *et al*. (2014) have reported that attenuation of GnRH and LH discharge during pregnancy in rats may be regulated by a persistent *Rfrp* mRNA expression and diminished *Kiss1* mRNA expression.

Expression and action of GnIH–GnIHR and KP-Kiss1r signaling in peripheral tissues implicated in energy homeostasis and reproduction

Careful analyses of the expression of components of GnIH–GnIHR and KP-Kiss1r signaling pathways show that these are highly active in two kinds of organs: i) those that are involved in the maintenance of metabolic homeostasis and ii) those that control reproduction. The pituitary, which is vital in the control of metabolic homeostasis and reproduction, highly expresses *Rfrp*, *GnIHR*, *Kiss1,* and *Kiss1r* (Lee *et al*. 1996, Muir *et al*. 2001, Ohtaki *et al*. 2001, Shahab *et al*. 2005, Ubuka *et al*. 2009*b*, Bentley *et al*. 2010, Smith *et al*. 2010*a*,*b*, Tsutsui *et al*. 2010, Wahab *et al*. 2011*a*, Cejudo Roman *et al*. 2012, Irfan *et al*. 2014, Osugi *et al*. 2014).

Furthermore, expression of some of these genes has been reported in the adipose tissue, adrenal gland, placenta, pancreas, and intestine (Lee *et al*. 1996, 1999, Ohtaki *et al*. 2001, Brown *et al*. 2008). These organs are crucial for the maintenance of metabolic homeostasis. Likewise, these genes are also expressed in the primary sex organs, ovaries, and testes, which drive reproduction (Ohtaki *et al*. 2001, Bentley *et al*. 2010, Li *et al*. 2012, Irfan *et al*. 2014). While the roles of GnIH–GnIHR and KP-Kiss1r in the functioning of these peripheral organs are not fully understood at present, the modulation of these organs' endocrine secretion by the administration of GnIH and KP suggests a significance of GnIH and KP expression in the periphery.

Infection/immune challenge induced malnutrition-related alterations in hypothalamic KP signaling

It is well known that infection/immune challenges cause an inhibition of the reproductive axis (Fig. 4) (Wahab *et al*. 2013*b*). It is also known that infection/immune challenges result in a kind of metabolic deficiency state by various mechanisms. These include an increase in energy expenditure concurrently with a decrease in food intake, an increase in secretion of catabolic hormones such as corticosteroids and catecholamines, stimulation of the anorexigenic (α-melanocyte-stimulating hormone and corticotropin-releasing hormone), and inhibition of orexigenic (agouti-related protein) hypothalamic neuropeptides by cytokines, as well as an increase in cytokine-induced thermogenesis (Beisel 1995, Wahab *et al*. 2013*b*). However, the mechanistic link between infection/immune challenges and reproduction is still poorly understood.

Lipopolysaccharide (LPS) administration has been shown to greatly decrease the hypothalamic levels of *Kiss1* mRNA and reduce systemic levels of LH in gonadectomized rats (Iwasa *et al*. 2008, 2014, Castellano *et al*. 2010*b*). The inhibitory effect of LPS on the expression of hypothalamic mRNA levels of *Kiss1* and systemic levels of LH is fully reversed by indomethacin, a nonsteroidal

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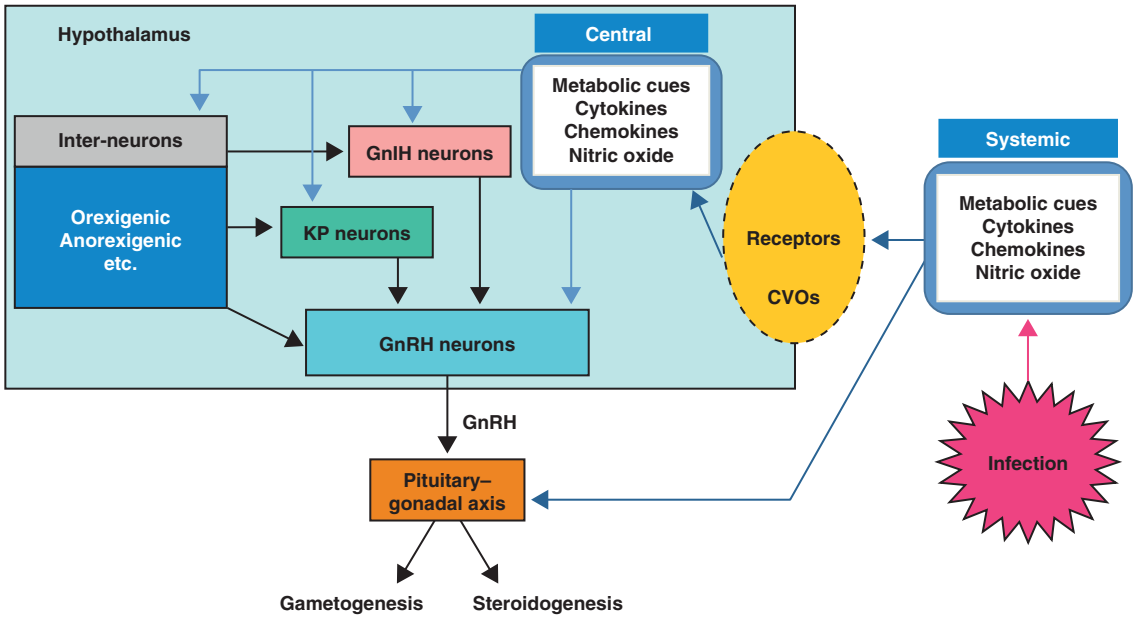

Figure 4
A schematic representation of a possible mechanism by which infection/immune challenges may affect the neuroendocrine network regulating reproductive functions. Infection/immune challenges cause changes in metabolic cues in addition to resulting in excessive release of cytokines, chemokines, and nitric oxide (NO). These factors enter the hypothalamus either directly or bind with its receptors on the projections of hypothalamic neurons in areas outside the blood–brain barrier such as circumventricular organs (CVO). Altered metabolic cues, cytokines, and NO can affect the activity of GnRH neurons directly as well as indirectly through KP and GnIH cells or via other interneurons. These signals can also act on pituitary gonadotropes to alter gonadotropin discharge. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-14-0688.

anti-inflammatory drug. Moreover, it was observed that exogenous KP injection greatly increased systemic levels of LH in LPS-injected animals just like placebo animals (Iwasa *et al.* 2008, 2014, Castellano *et al.* 2010*b*). These results suggest that KP-containing cells are highly sensitive to immune/inflammatory challenges and convey these signals to the network of GnRH-containing neurons in the hypothalamus. There is no literature on the effect of infection/immune challenges on GnIH in mammals. However, a recent study in birds has reported no effect of LPS on hypothalamic GnIH expression even though the expression of GnRH was significantly lowered in these birds (Lopes *et al.* 2012).

Future perspective

Recent findings of alterations in adiposity, body weight, glucose homeostasis, and metabolism, particularly in adult females in the absence of KP signaling, has point to the major importance of this signaling circuitry, especially in females (Tolson *et al.* 2014). However, further studies are needed to clarify the causes of the differences in KP signaling for these metabolic parameters between males and females.

The mechanism underlying the human post-ejaculatory refractory period is still not fully clear. Very recently, Pazhoohi & Saied Salehi (2013) have proposed an hypothesis about the possible involvement of GnIH in the post-ejaculatory refractory period after orgasm. It would be worthwhile to test this hypothesis in experimental models. Many studies have described the involvement of oxytocin and prolactin in the post-ejaculatory refractory period (Ogawa *et al.* 1980, Krüger *et al.* 2003, Corona *et al.* 2012). The recent observations that GnIH modulates the secretion of oxytocin (Kaewwongse *et al.* 2011) further strengthen a possible involvement of this hormone in the post-ejaculatory refractory period. A comparison of GnIH and KP expression before, between, and after orgasm in animal models is required to ascertain the involvement of these hormones. Moreover, analysis of the metabolic cues in this setup and comparison of the expression of GnIH and KP would also be worth considering.

The metabolic significance of such a huge rise in systemic KP concentration during pregnancy (Horikoshi *et al.* 2003, Reynolds *et al.* 2009) remains largely unknown. Indeed, further research studies are needed to unveil any possible involvement of KP in pregnancy-related metabolic perturbations.

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Although KP neurons express *LepR*, and *Kiss1* expression is modulated by leptin administration in conditions of energy imbalance, recent evidence has suggested an indirect action of leptin on KP neurons because leptin administration did not alter STAT3 expression in these neurons (Louis *et al*. 2011). Therefore, more studies are needed to determine the contributions of other leptin-activated hypothalamic signaling pathways to changes in KP neuronal activities. Moreover, Donato *et al*. (2011) demonstrated that specific deletion of *LepR* from KP cells in the hypothalamus did not affect LH secretion. In the same vein, re-expression of *LepR* in *LepR*-null mice also did not improve infertility phenotype in animals (Cravo *et al*. 2013). These findings suggest that KP signaling is not crucial for linking leptin action with reproduction. Therefore, it will be interesting to know whether leptin administration would reverse metabolic deficiency-induced reproductive quiescence after the KP-Kiss1r signaling pathway blockade or ablation. As GnRH neurons do not express *LepR*, leptin acts indirectly via interneurons on these central regulators of the reproduction (Quennell *et al*. 2009, Donato *et al*. 2011, Zuure *et al*. 2013, Bellefontaine *et al*. 2014, Martin *et al*. 2014). Indeed, in the available literature, many hypothalamic neuronal pathways have been described to mediate leptin action on GnRH (Quennell *et al*. 2009, Donato *et al*. 2011, Zuure *et al*. 2013, Bellefontaine *et al*. 2014, Martin *et al*. 2014). Besides central hypothalamic actions, a number of studies suggest that leptin can also act directly on pituitary gonadotropes to modulate LH secretion (Crane *et al*. 2007, Akhter *et al*. 2014). GnIH can also act on pituitary gonadotropes to inhibit LH secretion (Ubuka *et al*. 2009*b*, 2014, McGuire & Bentley 2010, Anjum *et al*. 2014), whether leptin modulates this direct action of GnIH on gonadotropes warrants further investigations.

Conclusion

Herein, we summarized compelling evidence which suggests an active involvement of KP and GnIH in the metabolic regulation of reproduction. GnIH and KP neurons are located in a strategic position in the hypothalamus, where they collect information regarding the body's current metabolic status from various peripheral and central sources (Fig. 2) (Qi *et al*. 2009, Castellano *et al*. 2010*a*, Sanchez-Garrido & Tena-Sempere 2013, Wahab *et al*. 2013*a*, Batool *et al*. 2014, Poling *et al*. 2014, Sabet Sarvestani *et al*. 2014) and communicate them to the hypothalamic neural circuits regulating the HPG axis. GnIH and KP can provide this relay of metabolic information both directly and indirectly. In the former case, GnIH and KP neurons perceive metabolic information from either metabolic cues or orexigenic and anorexigenic neurons and then pass them to the GnRH neuronal network. In the latter case, GnIH and KP neurons obtain data regarding the body's metabolic status from circulating metabolic cues and neurons of feeding and satiety centers and then transfers it via interneurons to the neuronal network.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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Author contribution statement
F W drew the figures and wrote the first draft of this review article. M S and R B edited and added to this review article. All authors read and approved the final version of the manuscript.

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