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

F Wahab, M Shahab and R Behr

Stem Cell Biology Unit, Leibniz Institute for Primate Research, German Primate Center, Kellnerweg 4, D-37077 Göttingen, Germany

1Laboratory of Reproductive Neuroendocrinology, Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Correspondence should be addressed to F Wahab
Email fwahab@dpz.eu

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

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. 2013a,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 1963a, Foster & Olster 1985, Cameron 1991, 1996). Food resumption regularizes the
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 (Batoool 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 (Batoool et al. 2010a, b, Sanchez-Garrido & Tena-Sempere 2013, Wahab et al. 2013a).

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. 2011a).

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, 2009a). 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. 2009b, 2014, Sari et al. 2009, McGuire & Bentley 2010, Anjum et al. 2014).
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, 2009b, 2012, Rizwan et al. 2012). In addition to its hypothalamic expression, GnIHR expression has been reported in the pituitary (Ubuka et al. 2009b) and the gonads (McGuire & Bentley 2010). The inhibitory effect of GnIHR on reproductive hormones secretion can be blocked by the GnIHR antagonist RF9 (Pineda et al. 2010a,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.

**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. (2011b), 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. 2005, 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. (2011b), 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,
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, 2005a, 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.


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. 2010a, b, Clarke & Caraty 2013, Kriegerfeld 2013, Sanchez-Garrido & Tena-Sempere 2013, Wahab et al. 2013a). 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 1963b, 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 anorexigenic 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. 2009a, b, Escobar et al. 2013, Sabet Sarvestani et al. 2014, Obga 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

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. 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 neuronal 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, dynorphin 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 et 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
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. 2010a).

During the prepubertal period, the body’s metabolic reserves exert a vital influence on the pubertal awakening of the HPG axis (Kennedy & Mitra 1963a, 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. 2010a, 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 1963a, 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 et al. 2013a,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. 2011a, 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 (Crand 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 (Crand 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...
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. 2011a).

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 GnIH 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, 2011c, 2013a, 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 LepRs (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. 2011a), 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
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). 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 sucking 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 sucking stimulus-related information to the GnRH neuronal network as shown in Fig. 3 (Smith et al. 2010a, Liu et al. 2014). An obvious increase in GnIH was reported in the hypothalamus of lactating

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

<table>
<thead>
<tr>
<th>Metabolic paradigm</th>
<th>Kiss1 mRNA</th>
<th>RFRP mRNA</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Fasting</td>
<td>–</td>
<td>+</td>
<td>Castellano et al. (2005), Wahab et al. (2011a) and Jahanara et al. (2014)</td>
</tr>
<tr>
<td>Lactation</td>
<td>–</td>
<td>+</td>
<td>Yousefabad et al. (2013) and Liu et al. (2014)</td>
</tr>
<tr>
<td>Diet-induced obesity</td>
<td>–, =</td>
<td>–</td>
<td>Quennell et al. (2011), Luque et al. (2007) and Rizwan et al. (2014)</td>
</tr>
<tr>
<td>Ob/Ob mice</td>
<td>–</td>
<td>–</td>
<td>Smith et al. (2006), Quennell et al. (2011) and Rizwan et al. (2014)</td>
</tr>
</tbody>
</table>

+, increase; –, decrease; =, no effect; ?, currently unknown.
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
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. 2009b, Bentley et al. 2010, Smith et al. 2010a,b, Tsutsui et al. 2010, Wahab et al. 2011a, 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. 2013b). 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. 2013b). 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. 2010b). 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
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. 2010b). 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.
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. 2009b, 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. 2010a, Sanchez-Garrido & Tena-Sempere 2013, Wahab et al. 2013a, 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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**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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