Comprehensive Review on Kisspeptin and Its Role in Reproductive Disorders

Holly Clarke, Waljit S. Dhillo, Channa N. Jayasena

Department of Investigative Medicine, Hammersmith Hospital, Imperial College London, London, UK

Kisspeptin has recently emerged as a key regulator of the mammalian reproductive axis. It is known that kisspeptin, acting centrally via the kisspeptin receptor, stimulates secretion of gonadotrophin releasing hormone (GnRH). Loss of kisspeptin signaling causes hypogonadotrophic hypogonadism in humans and other mammals. Kisspeptin interacts with other neuropeptides such as neurokinin B and dynorphin, to regulate GnRH pulse generation. In addition, a growing body of evidence suggests that kisspeptin signaling be regulated by nutritional status and stress. Kisspeptin may also represent a novel potential therapeutic target in the treatment of fertility disorders. Early human studies suggest that peripheral exogenous kisspeptin administration stimulates gonadotrophin release in healthy adults and in patients with certain forms of infertility. This review aims to concisely summarize what is known about kisspeptin as a regulator of reproductive function, and provide an update on recent advances within this field.

Keywords: Kisspeptins; Fertility; Hypothalamus; Gonadotropin-releasing hormone; Gonadotrophins

INTRODUCTION

Eleven years ago inactivating mutations in the gene encoding kisspeptin and its receptor were first observed to cause infertility. Research has since focused on delineating the exact role and mechanisms underlying the role of kisspeptin in reproduction. It is now widely accepted that kisspeptin, acting via the kisspeptin receptor, is a critical regulator of the reproductive axis by stimulating hypothalamic gonadotrophin releasing hormone (GnRH) release. In recent years, two other neuropeptides (neurokinin B [NKB] and dynorphin [DYN]) have shared the spotlight with kisspeptin as key hypothalamic regulators of reproductive function, and are thought to be co-secreted with kisspeptin to regulate GnRH secretion. More recently, studies have suggested that kisspeptin may also have direct gonadal effects and interact with metabolic pathways. Aided by increasing numbers of studies in humans, we are also beginning to define a potential therapeutic role for kisspeptin in treating certain forms of infertility. This review aims to summarize what is known about kisspeptin as a regulator of reproduction and provide an update on recent advances within this field.
DISCOVERY OF KISSPEPTIN

Kisspeptin was first discovered in 1996 as a metastasis inhibitor in melanoma cell lines [1]. Kisspeptin is actually a family of peptides derived from the KISS1/kiss1 gene with structural similarity, forming from differential proteolysis of a common precursor, prepro-kisspeptin. Kisspeptin peptides are classified as an RF amide peptide family i.e., neuroactive peptides with characteristic Arg-Phe-NH2 motif [2]. The most abundant kisspeptin in the human circulation is kisspeptin-54, which can be further cleaved to 14, 13, and 10 amino acid peptides [3].

THE KISSPEPTIN RECEPTOR

The kisspeptin receptor was discovered 4 years later than kisspeptin, and was originally known as GPR54 [4]. It is a member of the rhodopsin family of G-protein-coupled receptors and is structurally similar to the galanin receptor [2,3,5]. When kisspeptin binds the receptor, phospholipase C is activated which recruits secondary intracellular messengers, inositol triphosphate and diacylglycerol, which in turn mediate intracellular calcium release and protein kinase C activation [6-8]. A recent study showed that the intracellular calcium release is biphasic, the first phase being rapid with the second phase being slower. The slower phase is maintained by internalization and recycling of the receptor to prevent desensitization [9].

ANATOMICAL DISTRIBUTION OF KISSPEPTIN

Kisspeptin expression was first demonstrated in high levels in the placenta [5,6], and has subsequently been observed in the testis, ovary, pancreas, and small intestine [5,10]. Central expression of kisspeptin and its receptor have been demonstrated in two major neuronal populations within the hypothalamus of rodents: in the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV) [11]. In humans and primates, kisspeptin mRNA is predominantly expressed within the infundibular nucleus (equivalent of the ARC in this order of mammals) [12].

SEXUAL DIMORPHISM OF KISSPEPTIN NEURONAL DISTRIBUTION

In rodents, the kisspeptin neurons of the AVPV appear to be sexually dimorphic, with many more neurons in females than in males [13,14]. More recent evidence also supports the possibility of sexually dimorphic kisspeptin neuron populations in the rostral periventricular area of the third ventricle (RP3V) and infundibulum of humans [15,16]. It has previously been observed that the increase in kisspeptin expression within the RP3V during pubertal development is dependant upon estradiol in female mice [17,18]. Furthermore, Clarkson et al. [19] recently observed that, in male mice, gonadectomy at postnatal day 20 resulted in a reduced number of kisspeptin immunoreactive (IR) neurons within the RP3V, which was restored by administration of both estradiol and testosterone.

KISSPEPTIN STIMULATES ENDOGENOUS GnRH TO ACTIVATE THE REPRODUCTIVE AXIS

Kisspeptin neurons exist in close apposition with GnRH neurons in the hypothalamus of a range of species [13,20], and GnRH neurons express the kisspeptin receptor [21,22]. Kisspeptin stimulates GnRH neurons leading to GnRH release in both in vitro and in vivo studies [7,23,24], an effect which is inhibited by the administration of GnRH antagonists [25]. Furthermore, kisspeptin administration both centrally and peripherally leads to an increase in circulating lutenizing hormone (LH) levels in both animal and human studies [11,26-28]. Expression of the kisspeptin receptor gene has been observed in both the ARC and AVPV. Kisspeptin neurons project to the cell bodies of GnRH neurons in the preoptic area, and to the median eminence, close to GnRH nerve endings [11,13,29]. Taken together, these data suggest that kisspeptin stimulates GnRH neurons in the hypothalamus to release GnRH into the hypothalamic-pituitary portal circulation, causing the release of gonadotrophs from the anterior pituitary [30]. A recent study suggests that ovariectomy may abolish the kisspeptin-induced GnRH release in pubertal monkeys, and estradiol replacement may result in partial recovery of kisspeptin-induced GnRH release [31]. These data suggest that kisspeptin requires estradiol to stimulate GnRH secretion.

KISSPEPTIN PLAYS A CRITICAL ROLE IN THE ONSET OF PUBERTY

In 2003 De Roux et al. [32] and Seminara et al. [33] discovered a number of mutations in the kisspeptin receptor gene in humans with congenital hypogonadotropic hypogonadism (CHH). These landmark findings have paved the way for a
number of other studies examining mutations in the human kisspeptin receptor [34-39]. The CHH phenotype has also been observed in patients with heterozygous kisspeptin receptor mutations [40], suggesting an integral role of kisspeptin in puberty. More recently, an inactivating mutation in the kisspeptin gene in humans with absent progression of puberty has also been reported [41].

The use of knockout mouse models has allowed more in-depth study into the exact mechanism and function of kisspeptin in sexual maturation. In 2003, Seminara et al. [33] first showed that kisspeptin receptor null mice displayed hypogonadotropic hypogonadism (HH), as suggested by low levels of circulating gonadotrophin hormones, with small testes in male mice and a delay in vaginal opening and an absence of follicular maturation in female mice. The administration of exogenous GnRH corrected the HH phenotype, which is consistent with the view that kisspeptin acts by stimulating endogenous GnRH. A number of subsequent studies have provided similar findings [42-44].

Kisspeptin expressing neurons in the AVPV of mice are only detectable from postnatal day 25, with peak adult levels being reached by the onset of puberty at day 31 [13]. Navarro et al. [45] administered central injections of kisspeptin to mice from postnatal day 26 to day 31, and observed precocious vaginal opening, increased uterine weight and raised plasma LH and estradiol levels relative to vehicle-treated controls, first implicating kisspeptin in the pathogenesis of precocious puberty. Four years later, Teles et al. [46] identified an activating autosomal dominant mutation in the kisspeptin receptor gene in a girl with precocious puberty. These studies paved the way for many others investigating the role of kisspeptin in the pathogenesis of precocious puberty.

Polymorphisms in the kisspeptin receptor gene have been associated with congenital precocious puberty (CPP). Ko et al. [47] studied patients with CPP and found a polymorphism in the kisspeptin receptor gene occurring less frequently in CPP compared with controls. By contrast, Silveira et al. [48] identified two different mutations in patients with CPP, resulting in kisspeptin which was more resistant to degradation when compared to wild type. Plasma levels of kisspeptin have been observed to be higher in a cohort of Korean girls with CPP versus prepubertal age-matched controls [49]. Furthermore, plasma kisspeptin levels measured after 6 months of treatment for girls with CPP were significantly reduced when compared with pre-treatment levels [50].

More recently, Rhie et al. [51] investigated sequence variations of the kisspeptin gene in a large Korean cohort with CPP. They found three different single-nucleotide polymorphisms which occurred at different rates between the CPP group versus control, including one which was suggested to provide a protective effect [51].

**KISSPEPTIN AS A REGULATOR OF SEASONAL REPRODUCTION**

Kisspeptin may also regulate seasonal reproduction in certain species. Increased hypothalamic kisspeptin expression has been reported in Syrian hamsters during long day conditions, associated with increased sexual activity [52]. Revel et al. [53] observed that administration of kisspeptin-10 to Syrian hamsters under photoinhibitory conditions restored testicular, and therefore reproductive, activity. Sheep are also known to be seasonal breeders, with increased reproductive activity during short days. Clarke et al. [54] observed increased ARC kisspeptin expression in ewes during short day conditions, but no change in kisspeptin expression levels in preoptic area. Conversely, during long day periods kisspeptin expression in the ARC of ewes is reduced [55]. Furthermore, kisspeptin administration in seasonally acyclic ewes induces ovulation [56]. More recently, it has been suggested that GnRH (and LH) responses to kisspeptin are greater in anestrous ewes compared with luteal phase ewes [57]. In addition, kisspeptin receptor expression on GnRH neurons was greater during the non-breeding season compared with the breeding season.

A recent study examined expression of kisspeptin, together with NKB and DYN, in Syrian hamsters. They observed that all three neuropeptides were down-regulated in the ARC under a short photoperiod [58]. Piekarski et al. [59] compared the effects of long and short day conditions, and pinealectomy, on hypothalamic kisspeptin expression in certain species. Increased hypothalamic kisspeptin expression has been reported in Syrian hamsters during long day conditions, associated with increased sexual activity [52]. Revel et al. [53] observed that administration of kisspeptin-10 to Syrian hamsters under photoinhibitory conditions restored testicular, and therefore reproductive, activity. Sheep are also known to be seasonal breeders, with increased reproductive activity during short days. Clarke et al. [54] observed increased ARC kisspeptin expression in ewes during short day conditions, but no change in kisspeptin expression levels in preoptic area. Conversely, during long day periods kisspeptin expression in the ARC of ewes is reduced [55]. Furthermore, kisspeptin administration in seasonally acyclic ewes induces ovulation [56]. More recently, it has been suggested that GnRH (and LH) responses to kisspeptin are greater in anestrous ewes compared with luteal phase ewes [57]. In addition, kisspeptin receptor expression on GnRH neurons was greater during the non-breeding season compared with the breeding season.

A recent study examined expression of kisspeptin, together with NKB and DYN, in Syrian hamsters. They observed that all three neuropeptides were down-regulated in the ARC under a short photoperiod [58]. Piekarski et al. [59] compared the effects of long and short day conditions, and pinealectomy, on hypothalamic kisspeptin expression in certain species. Increased hypothalamic kisspeptin expression has been reported in Syrian hamsters during long day conditions, associated with increased sexual activity [52]. Revel et al. [53] observed that administration of kisspeptin-10 to Syrian hamsters under photoinhibitory conditions restored testicular, and therefore reproductive, activity. Sheep are also known to be seasonal breeders, with increased reproductive activity during short days. Clarke et al. [54] observed increased ARC kisspeptin expression in ewes during short day conditions, but no change in kisspeptin expression levels in preoptic area. Conversely, during long day periods kisspeptin expression in the ARC of ewes is reduced [55]. Furthermore, kisspeptin administration in seasonally acyclic ewes induces ovulation [56]. More recently, it has been suggested that GnRH (and LH) responses to kisspeptin are greater in anestrous ewes compared with luteal phase ewes [57]. In addition, kisspeptin receptor expression on GnRH neurons was greater during the non-breeding season compared with the breeding season.

**EMERGENCE OF THE KISSPEPTIN/NEUROKININ B/DYNORPHIN NEURONAL CONCEPT**

In more recent years, two other neuropeptides have come under the spotlight for their role in regulating reproduction: NKB and DYN. NKB is known for its role in steroid feedback control of GnRH release. It was recently discovered that, like kis-
Kisspeptin and Reproduction

Kisspeptin and Reproduction

Speeriptin, mutations in the gene encoding NKB, tachykinin 3 (TAC3), or its receptor (TACR3) leads to hypogonadism in humans [60,61]. DYN is an endogenous opioid peptide, which acts primarily through the $\alpha$-opioid receptor (KOR) [62]. DYN is known to regulate progesterone-mediated negative feedback on GnRH release [63]. In 2007, it was first discovered that these three neuropeptides are colocalised in hypothalamic neurons of the ARC in sheep [64]. Co-expression has also been demonstrated in rats [65], mice [66], goats [67], and humans [68,69]. Preservation of this subpopulation of neurons (subsequently named kisspeptin/neurokinin B/dynorphin [KNDy] neurons [70]) across several mammalian species suggests an integrated regulatory effect on GnRH release.

Numerous studies have provided anatomical evidence for a regulatory effect of KNDy neurons on GnRH release by demonstrating projections to GnRH neurons [29,71,72]. However, the precise role and intricate interactions of these neuropeptides in the regulation of reproduction is the subject of on-going research. It is known that kisspeptin stimulates LH release via GnRH neurons, whereas DYN inhibits GnRH pulse frequency [73]. Current models suggest that kisspeptin may trigger GnRH pulses, and DYN may terminate GnRH pulses [74]. Little expression of KOR is observed in GnRH neurons [75,76], it has therefore been proposed that DYN may act in an autocrine or paracrine manner to negatively regulate KNDy neurons which express KOR [66]. The role of NKB in GnRH pulse regulation remains controversial. The first study investigating the effects of NKB on LH release found that NKB receptor agonism resulted in suppression of LH release in ovariectomised, oestrogen replaced rats [77]. However, other animal studies suggest that NKB receptor agonism stimulates LH release [66,78-80]. Recent work by Jayasena et al. [81] observed that peripheral administration of NKB in healthy humans had no effect on gonadotropin release. It has been proposed that the differential effects of NK3R agonism observed may arise due to differences in steroid hormone milieu during NKB administration. A recent model, proposed by Grachev et al. [82] incorporates recent data regarding the effects of senktide (a NK3R agonist) in both ovariectomised and intact female rats. It suggests that in a hyperoestrogenic environment, NKB acts via DYN/KOR signaling to suppress LH pulses [83,84], whereas in intact prepubertal rats, NKB upregulates kisspeptin-induced LH pulses [85] and increases LH levels in diestrous rats [83].

Recent work by Young et al. [86] found that continuous kisspeptin infusion restored pulsatile LH secretion in humans with NKB or NK3R inactivating mutations causing infertility, providing strong evidence to suggest that NKB acts through kisspeptin to modulate downstream effects on GnRH secretion.

Some studies, however, have challenged the concept that a single population of neurons coexpress kisspeptin, NKB and DYN. Hrabovszky et al. [87] recently suggested that, in young human males, there is relatively little co-expression of DYN in neurons expressing kisspeptin and NKB. In addition, True et al. [88] did not observe co-expression of the three neuropeptides in rats.

**KISSPEPTIN REGULATES GONADAL STEROID FEEDBACK TO THE HYPOTHALAMUS**

It is well known that steroid hormones produced by the gonads exert feedback signaling to the hypothalamus to regulate GnRH production and release. Estrogen receptors (ERs) are transcription factors which exist as two isoforms: ER$\alpha$ and ER$\beta$. Estrogen is known to exert its positive feedback via centrally located ER$\alpha$ to induce the LH surge [89,90]. However, GnRH neurons lack the ER$\alpha$ in rats [91], suggesting the involvement of an intermediary neuronal pathway. Key work by Smith et al. [92] in 2006 investigated the potential role of kisspeptin in mediating the estrogen-induced LH surge. They observed that kisspeptin expression in the AVPV of rats was highest during the evening of proestrus, whereas expression levels in the ARC were at their lowest during this time. Kisspeptin expression was increased in the AVPV at the time of an estrogen and progesterone-induced LH surge in ovariectomized rats, whereas kisspeptin expression in the ARC was at its lowest during this time. Furthermore, kisspeptin neurons in the AVPV co-express the immediate early gene Fos at the time of the LH surge, whereas minimal Fos expression was observed on diestrous. In contrast, kisspeptin neurons in the ARC did not express Fos during the LH surge or on diestrous. Lastly, they observed that most kisspeptin neurons in both the AVPV and ARC express the ER$\alpha$. Taken together, these data suggest that kisspeptin neurons in the AVPV play a role in mediating estrogen signaling to generate the preovulatory LH surge in rats [92].

A number of other studies have investigated the role of kisspeptin signaling in the LH surge. Exogenous kisspeptin administration has been observed to potently induce LH secretion resulting in ovulation in rats [93,94]. Furthermore, the estrogen-induced preovulatory surge is inhibited by the administration of anti-kisspeptin antibodies in rats [95,96]. Clarkson
et al. [97] observed that, in knockout mouse models, kisspeptin receptor signaling was critical for the LH surge and subsequent ovulation. In contrast, kisspeptin receptor knockout mice created by Dungan et al. [98] underwent an estrogen-induced LH surge, suggesting that kisspeptin may not be critical to this process.

More recently, Tomikawa et al. [99] examined the epigenetic regulation of kisspeptin gene expression mediating estrogen-positive feedback action in mice. They observed that the histone of the kisspeptin gene locus in the AVPV was highly acetylated, and the ERα was highly recruited at the region by estrogen, whereas the same locus in the ARC showed histone deacetylation in response to estrogen. This suggests that epigenetic regulation of kisspeptin may regulate kisspeptin expression in the AVPV in response to estrogen, and underlies the estrogen positive feedback resulting in the LH surge [99].

### POTENTIALLY DIRECT GONADAL EFFECTS OF KISSPEPTIN

Whilst the central effects of kisspeptin are increasingly well described, it remains possible that direct gonadal effects of kisspeptin also exist. In 2004, Terao et al. [100] first observed expression of the genes encoding kisspeptin and its receptor in rat ovaries, which has subsequently been demonstrated in primate and human ovaries [10,101]. Furthermore, Castellano et al. [102] observed that ovarian expression of kisspeptin, and kisspeptin IR is cycle dependent in rats.

More recently, a study was able to provide functional evidence of a direct effect of kisspeptin on ovaries in mice, independent of its central effects via gonadotrophins. Gaytan et al. [103] observed that both kisspeptin receptor null and haplo-insufficient mice had premature ovarian failure (POF), associated with decreased ovarian kisspeptin receptor expression. In the context of preserved levels of circulating gonadotrophins, this implies a direct interaction between kisspeptin and the ovaries may contribute to the pathogenesis of POF [103]. Furthermore, Dorfman et al. [104] recently demonstrated that neurotrophin signaling via the NTRK2 receptor (essential for oocyte maturation during the preovulatory LH surge) is dependent upon kisspeptin receptor signaling using knockout mouse models. They suggest that both signaling pathways are required for oocyte survival and follicular integrity in the adult ovary [104].

The genes encoding kisspeptin and its receptor are expressed in both human and rodent testes [3,5,100,105]. Irfan et al. [106] recently examined the effects of kisspeptin on the testes in adult male monkeys. Kisspeptin administration enhanced human chorionic gonadotrophin (hCG) stimulated testosterone release in acyline treated monkeys, but had no effect on its own in acyline treated monkeys. They suggest that kisspeptin may potentiate the effect of hCG on testosterone release from the gonads via a novel peripheral pathway [106].

Pinto et al. [107] detected kisspeptin and its receptor in human spermatozoa. They observed that exposure of human spermatozoa to kisspeptin resulted in a biphasic rise in intracellular calcium, with associated increased motility [107]. Furthermore, Hsu et al. [108] recently suggested that kisspeptin modulates the fertilization capacity of mouse spermatozoa by promoting capacitation, and that administration of a kisspeptin antagonist reduced fertilization rates of spermatozoa in rats. The biological significance of these findings are currently unclear. However, taken together these data suggest that kisspeptin may act peripherally to regulate gonadal function in both males and females.

### ROLE OF KISSPEPTIN IN PREGNANCY AND IMPLANTATION

The highest levels of peripheral kisspeptin expression in the body have been found in the syncytiotrophoblast cells of the placenta [109,110]. Circulating levels of kisspeptin have been shown increase with gestation in humans, with levels in late pregnancy rising to up to 7,000 times greater than in non-pregnant controls [111,112]. Levels of kisspeptin receptor expression are increased in placental tissue with gestational trophoblastic disease when compared with normal placental tissue [113]. Furthermore, plasma kisspeptin IR is raised in patients with gestational trophoblastic neoplasia when compared with non-pregnant controls, and falls during and after chemotherapy [114]. The precise function of kisspeptin in these instances is unclear, although it has been speculated that it may act to regulate trophoblast cell invasion [111]. Thus, studies have proceeded to investigate the potential link between kisspeptin levels and placental dysfunction such as pre-eclampsia [115], and intrauterine growth restriction [116]. Cetkovic et al. [117] found plasma kisspeptin levels to be significantly lower in pregnant women with diabetes mellitus type 1, gestational diabetes, hypertension, pulmonary embolism, and placental dysfunction compared with healthy pregnant controls.

Park et al. [118] first suggested a link between kisspeptin and miscarriage. They observed that levels of placental kisspeptin expression are lower in women with recurrent miscarriage
when compared with placental tissue in electively terminated pregnancies, although no matching for gestational age was performed [118]. Furthermore, maternal plasma kisspeptin-10 levels are lower in women with early pregnancy bleeding, suggesting a possible association with abortus imminens [119]. Jayasena et al. [112] recently observed that plasma kisspeptin levels were significantly lower during the first trimester of pregnancy in women who went on to suffer miscarriage compared with healthy pregnancies, and suggest that kisspeptin may provide a potential novel marker for identifying asymptomatic pregnant women at increased risk of miscarriage.

**A REGULATORY ROLE FOR KISSPEPTIN IN NUTRITION AND FERTILITY**

It is well known that body weight affects fertility. The signals regulating body weight and energy expenditure have been extensively studied in recent years. Leptin is a peptide hormone secreted by adipocytes [120]. Deficiency of leptin results in delayed puberty and hypogonadotropic hypogonadism in mice [121] and humans [122]. Furthermore, leptin administration reverses the infertility associated with leptin deficiency [121, 123]. Subsequently it was hypothesised that leptin may constitute a link between nutrition and fertility. However, GnRH neurons lack receptors for many of the major metabolic signaling peptides, including insulin and leptin [124].

Kisspeptin is implicated as an intermediary between leptin signaling and GnRH function. Kisspeptin neurons express the leptin receptor, and *Ob/Ob* mice have reduced ARC levels of kisspeptin mRNA compared with wild type controls [125]. Furthermore, kisspeptin expression is increased following exogenous leptin administration [125]. Fasting has been shown to reduce hypothalamic kisspeptin mRNA and delay the onset of puberty in rats. In addition, central administration of kisspeptin to chronically undernourished prepubertal rats restored parameters of delayed puberty [126]. However, Donato et al. [127] demonstrated that specific knockout of the leptin receptor in kisspeptin neurons did not inhibit reproduction in rodents, suggesting that kisspeptin is not a critical component in the effect of leptin on reproduction.

Studies have also examined possible indirect actions by which leptin may regulate kisspeptin neurons in the hypothalamus. Neuropeptide Y (NPY) is an orexigenic peptide known to increase food intake. Pro-opiomelanocortin (POMC) is a precursor of α-melanocyte-stimulating hormone (α-MSH), known for its anorectic effects. Neurons expressing NPY and POMC have been shown to be in close apposition with kisspeptin neurons in the ARC [128]. Furthermore, central administration of an α-MSH agonist results in increased kisspeptin mRNA in the preoptic area and increased plasma LH levels [129].

Mammalian target of rapamycin protein (mTOR) is a key player in the regulation of energy homeostasis, acting to reduce cell growth and differentiation in undernutrition [130]. A link between mTOR and kisspeptin was suggested when antagonism of mTOR by rapamycin led to reduced kisspeptin expression in the ARC and reduced plasma LH levels [131].

Martin et al. [132] further examined the neuronal pathways mediating the effects of leptin on fertility, by creating mice with targeted deletions of GABAergic (predominantly inhibitory) neurons, and glutaminergic (excitatory) first order neurons. They found that GABAergic KO mice had delayed puberty and reduced parameters of reproductive function, whilst glutaminergic KO mice had normal pubertal onset and reproductive function. Furthermore, GABAergic KO mice had reduced levels of kisspeptin mRNA in the ARC compared with glutaminergic KO and wild type mice, with preserved GnRH and gonadotroph response to central administration of kisspeptin-10 [132]. These data suggest that leptin-responsive GABAergic neurons may convey signals of energy balance via kisspeptin neurons to regulate reproductive function. A recent study has also demonstrated that a subset of neurons expressing kisspeptin and NKB co-express the anorectic hypothalamic peptide cocaine and amphetamine regulated transcript in the infundibulum of postmenopausal women [69].

Evans et al. [133] investigated the relationship between insulin and kisspeptin signaling in the regulation of reproductive function. Using dual-label immunohistochemistry they found that 5% of kisspeptin IR cells express the insulin receptor. Furthermore, kisspeptin IR cell activation was not detected in response to insulin administration at physiological levels. Using kisspeptin-specific insulin receptor knockout mice (KIRKO) they also failed to observe any difference in the onset of puberty, estrous cyclicity or reproductive competency in KIRKO mice compared with wild type controls, suggesting that direct insulin signaling to kisspeptin neurons is not a critical pathway in the regulation of reproduction [133]. Qiu et al. [134] also investigated mice lacking insulin receptors in kisspeptin neurons. In the knockout mice, females had delayed vaginal opening and first estrus, and males had delayed sexual maturation compared with wild type controls. Both male and female knockout mice also had reduced LH levels in early puberty compared with wild type controls. However, no difference in

---

Copyright © 2015 Korean Endocrine Society
adult reproductive capacity was observed between knockouts and controls [134]. These data suggest that impaired insulin signaling via kisspeptin neurons delays the onset of puberty but does not affect adult fertility.

Another study investigated the effects of kisspeptin administration to fasted monkeys. They observed that monkeys fasted for 12, 18, and 24 hours all maintained testosterone release in response to intravenous kisspeptin, although the mean testosterone level at 3 hours postinjection was lower in the 18 and 24 hours fasted group compared with the 12 hours fasted group and fed controls. Furthermore, prolonged fasting (18 and 24 hours) resulted in a delayed initial testosterone rise in response to kisspeptin injection [135]. These results suggest that fasting-induced suppression of the reproductive axis may involve attenuated responsiveness to endogenous kisspeptin, although the exact mechanism requires further validation.

Sanchez-Garrido et al. [136] studied the effects of a high fat diet (HFD) on both metabolic and reproductive parameters in adolescent and adult male rats. They found that HFD rats, in addition to increased body weight and impaired glucose tolerance, had reduced testosterone levels, decreased hypothalamic kisspeptin receptor expression and decreased LH responsiveness to kisspeptin [136].

Tolson et al. [137] recently made the striking observation that kisspeptin receptor knockout female mice had increased body weight, adiposity, and leptin levels, and reduced glucose tolerance compared with wild type controls. Moreover, kisspeptin receptor knockout males showed no difference in body weight or glucose tolerance compared with controls. In females, the effect of kisspeptin was shown to be independent to that of sex steroids, as the phenotype persisted in knockout ovariectomised mice, and was absent in ovariectomised wild type controls [137]. These data suggest a sexually dimorphic effect of kisspeptin signaling, acting independently of sex steroids, to regulate body weight and glucose metabolism, although more work is needed to further explore these findings.

A recent study by Song et al. [138] further investigated the possible interaction between kisspeptin and glucose metabolism in mice. It has been suggested that increased glucagon secretion occurs prior to islet cell dysfunction in the pathogenesis of type 2 diabetes mellitus (T2DM) [139]. Song et al. [138] observed that glucagon stimulates hepatic kisspeptin production, which resulted in reduced glucose-stimulated insulin secretion (GSIS) from pancreatic islet β-cells. They also observed that synthetic kisspeptin administration led to reduced GSIS. Both humans and mice with T2DM were observed to have increased serum kisspeptin levels and increased hepatic kisspeptin expression. Lastly, they observed that specific knockout of hepatic kisspeptin in diabetic mice resulted in improved GSIS and glycaemic control [138]. Taken together, these data suggest that increased levels of glucagon may act via kisspeptin to impair GSIS in the pathogenesis of T2DM.

In summary, numerous studies have investigated the role of kisspeptin as an intermediary signal between nutrition and reproduction. There is anatomical evidence to suggest both direct and indirect signaling pathways between leptin and kisspeptin, although loss of this pathway appears not to critically impair reproductive function. Similarly, loss of insulin receptors in kisspeptin neurons did not impair adult reproductive capacity but did appear to delay the onset of puberty in mice. Hepatic kisspeptin may also act as an intermediary signal in the pathogenesis of impaired glycaemia.

**KISSPEPTIN AND STRESS**

Stress is known to inhibit reproductive function by suppressing GnRH release. Although the exact mechanisms underlying this profound effect remain unclear, the hypothalamic neuropeptide corticotrophin releasing factor (CRF) has been implicated [140,141]. Kinsey-Jones et al. [142] observed that expression of kisspeptin and its receptor is reduced in the ARC and medial preoptic area (mPOA) of mice in response to central injection of CRF. Reduced kisspeptin and kisspeptin expression was also observed in response to other stressors including restraint, insulin-induced hypoglycaemia and lipopolysaccharide (LPS) [142], suggesting that kisspeptin may contribute to stress-induced suppression of reproductive function.

LPS is commonly used to mimic immune stress as a model in the investigation of stress-induced suppression of reproductive function. LPS is known to reduce GnRH secretion in several mammalian species [143-145]. Knox et al. [146] observed that neonatal exposure to LPS caused delayed puberty and decreased kisspeptin mRNA in the mPOA of female rats. Furthermore, Iwasa et al. [147] recently demonstrated that intraperitoneal administration of high dose LPS in both ovariectomized and gonadal intact female rats led to decreased plasma LH levels and decreased hypothalamic kisspeptin and GnRH mRNA levels. They suggest that there is a steroid-independent role of kisspeptin in mediating stress-induced suppression of reproductive function [147].
POTENTIAL THERAPEUTIC APPLICATIONS OF KISSPEPTIN

Understanding the role and interactions of kisspeptin in the reproductive system is allowing us to identify a number of potential targets in the treatment of subfertility and other associated disorders of reproduction. Although kisspeptin primarily acts centrally to regulate reproduction, peripheral administration of kisspeptin has been shown to stimulate GnRH release in several animal studies [26,93], and subsequently in human studies [27,148,149] with no reported adverse effects. This has opened up the possibility of manipulating kisspeptin signaling in disorders related to both decreased GnRH signaling e.g., HH, and in disorders where the reproductive axis needs to be suppressed e.g., hormone sensitive cancers.

Human studies investigated the effects of exogenous kisspeptin on LH secretion. In 2005 Dhill et al. [27] observed that intravenous infusion of kisspeptin in healthy male subjects resulted in increased plasma gonadotrophin and testosterone levels. In 2007 the same group observed that subcutaneous kisspeptin injection in healthy pre-menopausal females led to increased plasma LH levels [28], an effect which was most pronounced in the preovulatory phase of the menstrual cycle. Jayasena et al. [150] examined the effects of kisspeptin administration in women with hypothalamic amenorrhoea. They observed that twice daily subcutaneous administration of kisspeptin led to an increase in plasma gonadotrophins [150], although this effect diminished after 2 weeks. However, twice weekly kisspeptin administration in the same cohort of women with hypothalamic amenorrhoea resulted in a sustained gonadotrophin response over an 8-week period [151]. Chan et al. [148] examined the effects of kisspeptin on endogenous GnRH pulse generation, as reflected by LH secretion in healthy human males. They observed that a single peripheral bolus of kisspeptin-10 induced an immediate LH pulse, irrespective of temporal relation to the previous endogenous pulse, and the mean amplitude of kisspeptin-induced LH pulses were greater than endogenous pulses. Furthermore, kisspeptin administration delayed the next endogenous LH pulse by roughly the normal interpulse interval, suggesting that kisspeptin might act to reset the GnRH pulse generator [148]. George et al. [149] observed that boluses of kisspeptin-10 potently induced LH secretion, and continuous infusion resulted in increased LH pulse frequency and size in healthy human men. Jayasena et al. [152] also observed that a single bolus of kisspeptin-54 increased LH pulsatility in healthy women, and kisspeptin-54 infusion increased LH pulsatility in women with hypothalamic amenorrhoea [153]. Furthermore, Young et al. [86] observed that continuous kisspeptin infusion restored LH pulsatility in patients with de-activating mutations in the genes encoding NKB or its receptor.

KISSPEPTIN AND THE FEMALE OVULATORY CYCLE

The effects of kisspeptin appear to vary at different stages in the menstrual cycle. The first study investigating this in humans found a maximal gonadotrophin response to exogenous kisspeptin during the preovulatory phase of the menstrual cycle [28]. Jayasena et al. [154] observed no gonadotrophin response to kisspeptin-10 administration in half of the women in the early follicular phase, and in all women in the luteal and preovulatory phase. Recently, Baba et al. [156] found that kisspeptin expression is increased in endometrial stromal cells through decidualization, suggesting a role for kisspeptin in preparing the endometrium for adequate placentation.

Several studies have shown that continuous kisspeptin administration causes desensitization in a range of species including humans [150,157,158]. As previously described, Jayasena et al. [150] investigated the effects of dose-interval kisspeptin-54 in women with hypothalamic amenorrhoea versus healthy female controls. In women with hypothalamic amenorrhoea, twice daily administration of kisspeptin-54 resulted in desensitization. However, healthy women remained responsive to twice weekly administration of kisspeptin. In contrast, George et al. [149] found no evidence of desensitization when kisspeptin-10 was infused continuously over 22.5 hours in healthy men, or with 11 hours infusions in hypotestosteronemic men with T2DM [159].

Animal data have suggested that kisspeptin may stimulate growth hormone (GH) and prolactin release from the anterior pituitary. Both in vitro [160,161] and in vivo [162,163] animal studies have suggested that kisspeptin may stimulate GH and prolactin release, although these findings were not replicated in monkeys [164]. Furthermore, a recent study investigated this possible effect in humans, and observed no change in mean serum GH, prolactin or thyroid-stimulating hormone levels in five healthy women following both acute and chronic kisspeptin-54 administration. In addition, no disturbance in GH pulse frequency or amplitude was observed [165]. We
therefore cannot exclude the possibility that kisspeptin stimulates nonreproductive pituitary hormones in humans, but would conclude that any effects are subtle. Prolactin is known to suppress gonadotrophin release [166]. Hyperprolactinaemia induced HH is a major cause of infertility, both physiological (during lactation), and pathological [167]. Kisspeptin neurons in the hypothalamus express the prolactin receptor, whereas GnRH neurons show minimal expression [168, 169]. Recent work by Araujo-Lopes et al. [170] demonstrated that, in ovariectomized rats, high prolactin levels suppressed kisspeptin expression in the ARC and subsequent LH release, suggesting that kisspeptin neurons may act as an intermediary signaling pathway in the prolactin-induced suppression of LH release. This may provide an additional therapeutic target in the development of new treatments for infertility caused by hyperprolactinaemia, which are resistant to first-line therapies.

With evidence from rodents and sheep that kisspeptin is a critical stimulus for the LH preovulatory surge, a recent study investigated the potential for kisspeptin to be used in women undergoing in vitro fertilization (IVF) therapy. Jayasena et al. [171] administered a single injection of kisspeptin-54 at differing doses to women undergoing IVF, following standard recombinant follicle-stimulating hormone and GnRH antagonist therapy. Egg maturation was observed in response to each tested dose of kisspeptin at 36 hours from administration. The mean number of mature eggs per patient increased in a dose-dependent manner [171]. Current practice most commonly uses hCG to trigger egg maturation [172], which acts directly on ovarian LH receptors to stimulate egg maturation. The use of hCG confers a risk of ovarian hyperstimulation syndrome (OHSS) due to sustained agonist activity compared with the endogenous LH surge, and a lack of negative feedback control. Thus, by stimulating endogenous GnRH and gonadotrophin release at physiological levels, kisspeptin use in IVF therapy may have reduced risk of OHSS, although comparison to existing therapies is required in larger studies.

CONCLUSIONS

It is widely accepted that kisspeptin plays an integral role in the regulation of reproduction. We are now forming a more in-depth understanding of the diverse and complex interactions in kisspeptin signaling. It appears that kisspeptin also participates in the translation of signals of nutritional state and stress into reproductive capacity via GnRH signaling. Furthermore, it is becoming increasingly apparent that kisspeptin acts together with NKB and DYN in a complex manner to precisely regulate GnRH pulse generation in response to dynamic changes in steroid hormone concentrations. Kisspeptin may represent a novel target in the treatment of fertility disorders. Thus far, results from human studies have been promising. In particular, the observations that kisspeptin increases LH pulsatility in women with hypothalamic amenorrhoea [150], and that kisspeptin induces egg maturation in a dose-dependent manner in women undergoing IVF treatment [171] provide hope that kisspeptin may be successfully used to develop new or improve existing fertility treatments. Research is also focusing on the use of prolonged kisspeptin agonism to induce testosterone suppression in the treatment of prostate cancer, with promising results from phase 1 clinical trials [173]. Furthermore, with the ability to manipulate the endogenous kisspeptin signaling pathway in therapeutics, it may be possible to reduce side-effects associated with current gold-standard therapies.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

The Section is funded by grants from the MRC, BBSRC, NIHR, an Integrative Mammalian Biology (IMB) Capacity Building Award, an FP7-HEALTH- 2009-241592 EuroCHIP grant and is supported by the NIHR Imperial Biomedical Research Centre Funding Scheme. W.S.D. is supported by an NIHR Career Development Fellowship.

REFERENCES

1. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. J Natl Cancer Inst 1996;88:1731-7.
2. Clements MK, McDonald TP, Wang R, Xie G, O’Dowd BF, George SR, Austin CP, Liu Q. FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54. Biochem Biophys Res Commun 2001;284:1189-93.
3. Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R,
Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 2001;276:34631-6.

4. Gottsch ML, Clifton DK, Steiner RA. From KISS1 to kisspeptins: an historical perspective and suggested nomenclature. Peptides 2009;30:4-9.

5. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kaneshashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 2001;411:613-7.

6. Muir AI, Chamberlain L, Elshourbagy NA, Michelovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Midleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J Biol Chem 2001;276:28969-75.

7. Liu X, Lee K, Herbison AE. Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. Endocrinology 2008;149:4605-14.

8. Constantin S, Caligioni CS, Stojilkovic S, Wray S. Kisspeptin-10 facilitates a plasma membrane-driven calcium oscillator in gonadotropin-releasing hormone-1 neurons. Endocrinology 2009;150:1400-12.

9. Min L, Soltis K, Reis AC, Xu S, Kuohung W, Jain M, Carroll RS, Kaiser UB. Dynamic kisspeptin receptor trafficking modulates kisspeptin-mediated calcium signaling. Mol Endocrinol 2014;28:16-27.

10. Gaytan F, Gaytan M, Castellano JM, Romero M, Roa J, Aparicio B, Garrido N, Sanchez-Criado JE, Millar RP, Pellicer A, Fraser HM, Tena-Sempere M. KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. Am J Physiol Endocrinol Metab 2009;296:E520-31.

11. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 2004;145:4073-7.

12. Rometo AM, Krajewski SJ, Voytko ML, Rance NE. Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. J Clin Endocrinol Metab 2007;92:2744-50.

13. Clarkson J, Herbison AE. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology 2006;147:5817-25.

14. Kauffman AS, Gottsch ML, Roa J, Byquist AC, Crown A, Clifton DK, Hoffman GE, Steiner RA, Tena-Sempere M. Sexual differentiation of Kiss1 gene expression in the brain of the rat. Endocrinology 2007;148:1774-83.

15. Hrabovszky E, Ciofi P, Vida B, Horvath MC, Keller E, Caraty A, Bloom SR, Gharai MA, Dhillon WS, Liposits Z, Kallo I. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur J Neurosci 2010;31:1984-98.

16. Hrabovszky E, Molnar CS, Sipos MT, Vida B, Ciofi P, Borsay BA, Sarkadi L, Herczeg L, Bloom SR, Gharai MA, Dhillon WS, Kallo I, Liposits Z. Sexual dimorphism of kisspeptin and neurokinin B immunoreactive neurons in the infundibular nucleus of aged men and women. Front Endocrinol (Lausanne) 2011;2:80.

17. Bakker J, Pierran S, Gonzalez-Martinez D. Effects of aromatase mutation (ArKO) on the sexual differentiation of kisspeptin neuronal numbers and their activation by same versus opposite sex urinary pheromones. Horm Behav 2010;57:390-5.

18. Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. Endocrinology 2009;150:3214-20.

19. Clarkson J, Shamas S, Mallinson S, Herbison AE. Gonadal steroid induction of kisspeptin peptide expression in the rostral periventricular area of the third ventricle during postnatal development in the male mouse. J Neuroendocrinol 2012;24:907-15.

20. Rance NE, Young WS 3rd, McMullen NT. Topography of neurons expressing luteinizing hormone-releasing hormone gene transcripts in the human hypothalamus and basal forebrain. J Comp Neurol 1994;339:573-86.

21. d’Anglemont de Tassigny X, Fagg LA, Carlson MB, Colledge WH. Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. Endocrinology 2008;149:3926-32.
tribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. Endocrinology 2010;151:312-21.

23. Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology 2004;80:264-72.

24. Novaira HJ, Ng Y, Wolfe A, Radovick S. Kisspeptin increases GnRH mRNA expression and secretion in GnRH secreting neuronal cell lines. Mol Cell Endocrinol 2009;311:126-34.

25. Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. Proc Natl Acad Sci U S A 2005;102:2129-34.

26. Thompson EL, Patterson M, Murphy KG, Smith KL, Dhilli WS, Todd JF, Ghatel AI, Bloom SR. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. J Neuroendocrinol 2004;16:850-8.

27. Dhilli WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatel MA, Bloom SR. Kisspeptin-54 stimulates the hypothalamic-pituitary-gonadal axis in human males. J Clin Endocrinol Metab 2005;90:6609-15.

28. Dhilli WS, Chaudhri OB, Thompson EL, Murphy KG, Patterson M, Ramachandran R, Nijher GK, Amber V, Kokkinos A, Donaldson M, Ghatel MA, Bloom SR. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. J Clin Endocrinol Metab 2007;92:3958-66.

29. Uenoyama Y, Inoue N, Pheng V, Homma T, Takase K, Yamada S, Ajiki K, Ichikawa M, Okamura H, Maeda KI, Tsukamura H. Ultrastructural evidence of kisspeptin-gonadotrophin-releasing hormone (GnRH) interaction in the median eminence of female rats: implication of axo-axonal regulation of GnRH release. J Neuroendocrinol 2011;23:863-70.

30. Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A 2005;102:1761-6.

31. Guerriero KA, Keen KL, Millar RP, Terasawa E. Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (Macaca mulatta): implication for the mechanism of puberty. Endocrinology 2012;153:825-36.
biallelic mutations in KISS1R (KISS1 receptor): clinical evaluation and molecular characterization of a novel mutation. PLoS One 2013;8:e53896.

40. Chan YM, Broder-Fingert S, Paraschos S, Lapatto R, Au M, Hughes V, Bianco SD, Min L, Plummer L, Cerrato F, De Guillebon A, Wu IH, Wahab F, Dwyer A, Kirsch S, Quinton R, Cheetham T, Ozata M, Ten S, Chanoine JP, Pitteloud N, Martin KA, Schiffmann R, Van der Kamp HJ, Nader S, Hall JE, Kaiser UB, Seminara SB. GnRH-deficient phenotypes in humans and mice with heterozygous variants in KISS1/Kiss1. J Clin Endocrinol Metab 2011;96:E1771-81.

41. Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 2012;366:629-35.

42. d’Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB, Aparicio SA, Colledge WH. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci U S A 2007;104:10714-9.

43. Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE, Seminara SB. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. Endocrinology 2007;148:4927-36.

44. Chan YM, Broder-Fingert S, Wong KM, Seminara SB. Kisspeptin/Gpr54-independent gonadotrophin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. J Neuroendocrinol 2009;21:1015-23.

45. Navarro VM, Fernandez-Fernandez R, Castellano JM, Roa J, Mayen A, Barreiro ML, Gaytan F, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. J Physiol 2004;561(Pt 2):379-86.

46. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358:709-15.

47. Ko JM, Lee HS, Hwang JS. KISS1 gene analysis in Korean girls with central precocious puberty: a polymorphism, p.P110T, suggested to exert a protective effect. Endocr J 2010;57:701-9.

48. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngar-ten M, Escobar ME, Arnhold IJ, Mendonca BB, Kaiser UB, Latronico AC. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab 2010;95:2276-80.

49. Rhie YJ, Lee KH, Eun SH, Choi BM, Chae HW, Kwon AR, Lee WJ, Kim JH, Kim HS. Serum kisspeptin levels in Korean girls with central precocious puberty. J Korean Med Sci 2011;26:927-31.

50. Demirbilek H, Gonc EN, Ozon A, Alikasifoglu A, Kandemir N. Evaluation of serum kisspeptin levels in girls in the diagnosis of central precocious puberty and in the assessment of pubertal suppression. J Pediatr Endocrinol Metab 2012;25:313-6.

51. Rhie YJ, Lee KH, Ko JM, Lee WJ, Kim JH, Kim HS. KISS1 gene polymorphisms in Korean girls with central precocious puberty. J Korean Med Sci 2014;29:1120-5.

52. Revel FG, Saboureau M, Masson-Pevet M, Pevet P, Mikkelsen JD, Simonneaux V. Kisspeptin mediates the photoperiodic control of reproduction in hamsters. Curr Biol 2006;16:1730-5.

53. Revel FG, Ansel L, Klosen P, Saboureau M, Pevet P, Mikkelsen JD, Simonneaux V. Kisspeptin: a key link to seasonal breeding. Rev Endocr Metab Disord 2007;8:57-65.

54. Clarke IJ, Smith JT, Caraty A, Goodman RL, Lehman MN. Kisspeptin and seasonality in sheep. Peptides 2009;30:154-63.

55. Smith JT, Clay CM, Caraty A, Clarke IJ. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 2007;148:1150-7.

56. Caraty A, Smith JT, Lomet D, Ben Said S, Morrissey A, Cognie J, Doughton B, Baril G, Briant C, Clarke IJ. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology 2007;148:5258-67.

57. Li Q, Roa A, Clarke IJ, Smith JT. Seasonal variation in the gonadotropin-releasing hormone response to kisspeptin in sheep: possible kisspeptin regulation of the kisspeptin receptor. Neuroendocrinology 2012;96:212-21.

58. Bartzen-Sprauer J, Klosen P, Ciofi P, Mikkelsen JD, Simonneaux V. Photoperiodic co-regulation of kisspeptin, neurokinin B and dynorphin in the hypothalamus of a seasonal rodent. J Neuroendocrinol 2014;26:510-20.

59. PiekarSKI DJ, Jarjissian SG, Perez L, Ahmad H, Dhawan N, Zucker I, Kriegsfeld LJ. Effects of pinealectomy and short day lengths on reproduction and neuronal RFRP-3, kiss-
peptin, and GnRH in female Turkish hamsters. J Biol Rhythms 2014;29:181-91.
60. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O’Rahilly S, Semple RK. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. Nat Genet 2009;41:354-8.
61. Guran T, Tolhurst G, Bereket A, Rocha N, Porter K, Turan S, Gribble FM, Kotan LD, Akcay T, Atay Z, Canan H, Serin A, O’Rahilly S, Reimann F, Semple RK, Topaloglu AK. Hypogonadotropic hypogonadism due to a novel missense mutation in the first extracellular loop of the neurokinin B receptor. J Clin Endocrinol Metab 2009;94:3633-9.
62. Wüster M, Schulz R, Herz A. Opiate activity and receptor selectivity of dynorphin1-13 and related peptides. Neurosci Lett 1980;20:79-83.
63. Goodman RL, Coolen LM, Anderson GM, Hardy SL, Valent M, Connors JM, Fitzgerald ME, Lehman MN. Evidence that dynorphin plays a major role in mediating progesterone negative feedback on gonadotropin-releasing hormone neurons in sheep. Endocrinology 2004;145:2959-67.
64. Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology 2009;149:5752-60.
65. Burke MC, Letts PA, Krajewski SJ, Rance NE. Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: morphologic evidence of interrelated function within the arcuate nucleus. J Comp Neurol 2006;498:712-26.
66. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci 2009;29:11859-66.
67. Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci 2010;30:3124-32.
68. Rance NE. Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. Peptides 2009;30:111-22.
69. Skrapis K, Borsay BA, Herczeg L, Ciofi P, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Hrabovszky E. Co-localization of cocaine-and amphetamine-regulated transcript with kisspeptin and neurokinin B in the human infundibular region. PLoS One 2014;9:e103977.
70. Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 2010;151:3479-89.
71. Krajewski SJ, Anderson MJ, Iles-Shih L, Chen KJ, Urbanski HF, Rance NE. Morphologic evidence that neurokinin B modulates gonadotropin-releasing hormone secretion via neurokinin 3 receptors in the rat median eminence. J Comp Neurol 2005;489:372-86.
72. Matsuyama S, Ohkura S, Mogi K, Wakabayashi Y, Mori Y, Tsukamura H, Maeda K, Ichikawa M, Okamura H. Morphological evidence for direct interaction between kisspeptin and gonadotropin-releasing hormone neurons at the median eminence of the male goat: an immunoelectron microscopic study. Neuroendocrinology 2011;94:323-32.
73. Gallo RV. Kappa-opioid receptor involvement in the regulation of pulsatile luteinizing hormone release during early pregnancy in the rat. J Neuroendocrinol 1990;2:685-91.
74. Goodman RL, Coolen LM, Lehman MN. A role for neurokinin B in pulsatile GnRH secretion in the ewe. Neuroendocrinology 2014;99:18-32.
75. Mitchell V, Prevot V, Jennes L, Aubert JP, Croix D, Beauvillain JC. Presence of mu and kappa opioid receptor mRNAs in galanin but not in GnRH neurons in the female rat. Neuroreport 1997;8:3167-72.
76. Sannella MI, Petersen SL. Dual label in situ hybridization studies provide evidence that luteinizing hormone-releasing hormone neurons do not synthesize messenger ribonucleic acid for mu, kappa, or delta opiate receptors. Endocrinology 1997;138:1667-72.
77. Sandoval-Guzman T, Rance NE. Central injection of senktide, an NK3 receptor agonist, or neuropeptide Y inhibits LH secretion and induces different patterns of Fos expression in the rat hypothalamus. Brain Res 2004;1026:307-12.
78. Navarro VM, Castellano JM, McConkey SM, Pineda R, Ruiz-Pino F, Pinilla L, Clifton DK, Tena-Sempere M, Steiner RA. Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat.
Am J Physiol Endocrinol Metab 2011;300:E202-10.
79. Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM. Neurokinin B stimulates GnRH release in the male monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology 2010;151:4494-503.
80. Ramaswamy S, Seminara SB, Plant TM. Evidence from the agonadal juvenile male rhesus monkey (Macaca mulatta) for the view that the action of neurokinin B to trigger gonadotropin-releasing hormone release is upstream from the kisspeptin receptor. Neuroendocrinology 2011;94:237-45.
81. Jayasena CN, Commins AN, De Silva A, Abbara A, Veldhuis JD, Nijhjer GM, Ganju-Dada Z, Vaa M, Stamp G, Ghatei MA, Bloom SR, Dhillo WS. Effects of neurokinin B administration on reproductive hormone secretion in healthy men and women. J Clin Endocrinol Metab 2014;99:E19-27.
82. Grachev P, Millar RP, O’Byrne KT. The role of neurokinin B signalling in reproductive neuroendocrinology. Neuroendocrinology 2014;99:7-17.
83. Kinsey-Jones JS, Grachev P, Li XF, Lin YS, Milligan SR, Lightman SL, O’Byrne KT. The inhibitory effects of neurokinin B on GnRH pulse generator frequency in the female rat. Endocrinology 2012;153:307-15.
84. Grachev P, Li XF, Kinsey-Jones JS, di Domenico AL, Millar RP, Lightman SL, O’Byrne KT. Suppression of the GnRH pulse generator by neurokinin B involves a kappa-opioid receptor-dependent mechanism. Endocrinology 2012;153:4894-904.
85. Grachev P, Li XF, Lin YS, Hu MH, Elsamani L, Paterson SJ, Millar RP, Lightman SL, O’Byrne KT. GPR54-dependent stimulation of luteinizing hormone secretion by neurokinin B in prepubertal rats. PLoS One 2012;7:e44344.
86. Young J, George JT, Tello JA, Franouc B, Bouligand J, Guiochon-Mantel A, Brailly-Tabard S, Anderson RA, Millar RP. Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiologic, pathophysiologic and therapeutic implications. Neuroendocrinology 2013;97:193-202.
87. Hrabovszky E, Sipos MT, Molnar CS, ciofi P, Borsay BA, Gergely P, Herczeg L, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z. Low degree of overlap between kisspeptin, neurokinin B, and dynorphin immunoreactivities in the infundibular nucleus of young male human subjects challenges the KNDy neuron concept. Endocrinology 2012;153:4978-89.
88. True C, Verma S, Grove KL, Smith MS. Cocaine- and amphetamine-regulated transcript is a potent stimulator of GnRH and kisspeptin cells and may contribute to negative energy balance-induced reproductive inhibition in females. Endocrinology 2013;154:2821-32.
89. Couse JF, Yates MM, Walker VR, Korach KS. Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. Mol Endocrinol 2003;17:1039-53.
90. Wintermantel TM, Campbell RE, Porteous R, Bock D, Grone HJ, Todman MG, Korach KS, Greiner E, Perez CA, Schutz G, Herbison AE. Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. Neuron 2006;52:271-80.
91. Herbison AE, Theodosius DT. Immunocytochemical identification of oestrogen receptors in preoptic neurones containing calcitonin gene-related peptide in the male and female rat. Neuroendocrinology 1992;56:761-4.
92. Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J Neurosci 2006;26:6687-94.
93. Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metestin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun 2004;320:E202-10.
94. Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P, Aguilar E, Dieguez C, Pinilla L, Tenasempere M. Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. Endocrinology 2005;146:156-63.
95. Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matozuno H, Uenoyma Y, Tsukamuru H, Inoue K, Maeda K. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. J Reprod Dev 2007;53:367-78.
96. Kinoshita M, Tsukamuru H, Adachi S, Matsui H, Uenoyma Y, Iwata K, Yamada S, Inoue K, Ohtaki T, Matsumoto H, Maeda K. Involvement of central metestin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology 2005;146:4431-6.
97. Clarkson J, d’Anglemont de Tassigny X, Moreno AS,
Colledge WH, Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. J Neurosci 2008;28:8691-7.

98. Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK, Clifton DK, Steiner RA. The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone. J Neurosci 2007;27:12088-95.

99. Tomikawa J, Uenoyama Y, Ozawa M, Fukunuma T, Takase K, Goto T, Abe H, Ieda N, Minabe S, Deura C, Inoue N, Sanbo M, Tomita K, Hirabayashi M, Tanaka S, Imamura T, Okamura H, Maeda K, Tsukamura H. Epigenetic regulation of Kiss1 gene expression mediating estrogen-positive feedback action in the mouse brain. Proc Natl Acad Sci U S A 2012;109:E1294-301.

100. Terao Y, Kuman S, Takatsu Y, Hattori M, Nishimura A, Ohtaki T, Shintani Y. Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta. Biochim Biophys Acta 2004;1678:102-10.

101. Cejudo Roman A, Pinto FM, Dorta I, Almeida TA, Hernandez M, Illanes M, Tena-Sempere M, Candenas L. Analysis of the expression of neurokinin B, kisspeptin, and their cognate receptors NK3R and KISS1R in the human female genital tract. Fertil Steril 2012;97:1213-9.

102. Castellano JM, Gaytan M, Roa J, Vigo E, Navarro VM, Bellido C, Dieguez C, Aguilar E, Sanchez-Criado JM, Pellicer A, Pinilla L, Gaytan F, Tena-Sempere M. Expression of KiSS-1 in rat ovary: putative local regulator of ovulation? Endocrinology 2006;147:4852-62.

103. Gaytan F, Garcia-Galiano D, Dorfman MD, Manfredi-Lozano M, Castellano JM, Dissen GA, Ojeda SR, Tena-Sempere M. Kisspeptin receptor haplo-insufficiency causes premature ovarian failure despite preserved gonadotropin secretion. Endocrinology 2014;155:3088-97.

104. Dorfman MD, Garcia-Rudaz C, Alderman Z, Kerr B, Lomniczi A, Dissen GA, Castellano JM, Garcia-Galiano D, Gaytan F, Xu B, Tena-Sempere M, Ojeda SR. Loss of Ntrk2/Kiss1r signaling in oocytes causes premature ovarian failure. Endocrinology 2014;155:3098-111.

105. Funes S, Hedrick JA, Vassileva G, Markowitz L, Abbonanzo S, Golovko A, Yang S, Monsma FJ, Gustafson EL. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun 2003;312:1357-63.

106. Irfan S, Ehmcke J, Wahab F, Shahab M, Schlatt S. Intra-testicular action of kisspeptin in rhesus monkey (Macaca mulatta). Andrologia 2014;46:610-7.

107. Pinto FM, Cejudo-Roman A, Ravina CG, Fernandez-Sanchez M, Martin-Lozano D, Illanes M, Tena-Sempere M, Candenas ML. Characterization of the kisspeptin system in human spermatozoa. Int J Androl 2012;35:63-73.

108. Hsu MC, Wang YJ, Lee YJ, Jong DS, Tsui KH, Chiu CH. Kisspeptin modulates fertilization capacity of mouse spermatozoa. Reproduction 2014;147:835-45.

109. Bilban M, Ghaflari-Tabrizi N, Hintzmann E, Bauer S, Molzer S, Zoratti C, Malli R, Sharabi A, Hiden U, Graier W, Knofer M, Andreae F, Wagner O, Quaranta V, Desoye G. Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. J Cell Sci 2004;117(Pt 8):1319-28.

110. Roseweir AK, Katz AA, Millar RP. Kisspeptin-10 inhibits cell migration in vitro via a receptor-GSK3 beta-FAK feedback loop in HTR8/SVneo cells. Placenta 2012;33:408-15.

111. Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S, Fujino M. Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. J Clin Endocrinol Metab 2003;88:914-9.

112. Jayasena CN, Abbara A, Izzo-Engbeaya C, Comninos AN, Harvey RA, Gonzalez Mafej F, Sarang Z, Ganiyu-Dada Z, Padilha AI, Dhanjal M, Williamson C, Regan L, Ghati MA, Bloom SR, Dhillon WS. Reduced levels of plasma kisspeptin during the antenatal booking visit are associated with increased risk of miscarriage. J Clin Endocrinol Metab 2014;99:E2652-60.

113. Janneau JL, Maldonado-Estrada J, Tachdjian G, Miran I, Motte N, Saulnier P, Sabourin JC, Cote JF, Simon B, Frydman R, Chaouat G, Bellett D. Transcriptional expression of genes involved in cell invasion and migration by normal and tumoral trophoblast cells. J Clin Endocrinol Metab 2002;87:5336-9.

114. Dhillon WS, Savage P, Murphy KG, Chaudhri OB, Patterson M, Nijher GM, Foggio VM, Dancey GS, Mitchell H, Seckl MJ, Ghati MA, Bloom SR. Plasma kisspeptin is raised in patients with gestational trophoblastic neoplasia and falls during treatment. Am J Physiol Endocrinol Metab 2006;291:E878-84.

115. Logie JJ, Denison FC, Riley SC, Ramaesh T, Forbes S, Norman JE, Reynolds RM. Evaluation of kisspeptin levels in obese pregnancy as a biomarker for pre-eclampsia. Clin Endocrinol (Oxf) 2012;76:887-93.
116. Smets EM, Deurloo KL, Go AT, van Vugt JM, Blankenstein MA, Oudejans CB. Decreased plasma levels of metatin in early pregnancy are associated with small for gestational age neonates. Prenat Diagn 2008;28:299-303.

117. Cetkovic A, Milicic D, Ljubic A, Patterson M, Ghatei M, Stamenkovic J, Nikolic-Djurovic M, Pekic S, Doknic M, Glisic A, Bloom S, Popovic V. Plasma kisspeptin levels in pregnancies with diabetes and hypertensive disease as a potential marker of placental dysfunction and adverse perinatal outcome. Endocr Res 2012;37:78-88.

118. Park DW, Lee SK, Hong SR, Han AR, Kwak-Kim J, Yang KM. Expression of Kisspeptin and its receptor GPR54 in the first trimester trophoblast of women with recurrent pregnancy loss. Am J Reprod Immunol 2012;67:132-9.

119. Kavvasoglu S, Ozkan ZS, Kumbak B, Simsek M, Ilhan N. Association of kisspeptin-10 levels with abortus imminens: a preliminary study. Arch Gynecol Obstet 2012;285:649-53.

120. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425-32.

121. Friedman JM. Positional cloning of the mouse obese gene. Cell 2000;103:253-62.

122. Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 2010;151:2233-43.

123. Backholer K, Smith J, Clarke IJ. Melanocortins may stimulate reproduction by activating orexin neurons in the dorsomedial hypothalamus and kisspeptin neurons in the preoptic area of the ewe. Endocrinology 2009;150:5488-97.

124. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell 2000;103:253-62.

125. Roa J, Garcia-Galiano D, Varela L, Sanchez-Garrido MA, Pineda R, Castellano JM, Ruiz-Pino F, Romero M, Aguilera E, Lopez M, Gaytan F, Dieguez C, Pinilla L, Tenasempere M. The mammalian target of rapamycin as a novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology 2009;150:5016-26.

126. Martin C, Navarro VM, Simavli S, Vong L, Carroll RS, Lowell BB, Kaiser UB. Leptin-responsive GABAergic neurons regulate fertility through pathways that result in reduced kisspeptinergic tone. J Neurosci 2014;34:6047-56.

127. Donato J Jr, Cravo RM, Frazao R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr, Coppari R, Zigman JM, Elmqquist JK, Elias CF. Leptin’s effect on puberty in mice is relayed by the ventral prefrontal hypothalamus and does not require signaling in Kiss1 neurons. J Clin Invest 2011;121:355-68.

128. Wahab F, Atika B, Huma T, Shahab M. Primate HPT axis response to the peripheral kisspeptin challenge under different time periods of food restriction in monkeys. Horm Metab Res 2014;46:187-92.

129. Sanchez-Garrido MA, Ruiz-Pino F, Manfredi-Lozano M, Leon S, Garcia-Galiano D, Castano JP, Luque RM, Romero-Ruiz A, Castellano JM, Dieguez C, Pinilla L, Tenasempere M. Obesity-induced hypogonadism in the male: premature reproductive neuroendocrine senescence and contribution of Kiss1-mediated mechanisms. Endocrinol-
137. Tolson KP, Garcia C, Yen S, Simonds S, Stefanidis A, Lawrence A, Smith JT, Kauffman AS. Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. J Clin Invest 2014;124:3075-9.

138. Song WJ, Mondal P, Wolfe A, Alonso LC, Stamateris R, Ong BW, Lim OC, Yang KS, Radovic S, Novaira HJ, Farber EA, Farber CR, Turner SD, Hussain MA. Glucagon regulates hepatic kisspeptin to impair insulin secretion. Cell Metab 2014;19:667-81.

139. D’Alessio D. The role of dysregulated glucagon secretion in type 2 diabetes. Diabetes Obes Metab 2011;13 Suppl 1:126-32.

140. Cates PS, Li XF, O’Byrne KT. The influence of 17beta-oestradiol on corticotrophin-releasing hormone induced suppression of luteinising hormone pulses and the role of CRH in hypoglycaemic stress-induced suppression of pulsatile LH secretion in the female rat. Stress 2004;7:113-8.

141. Li XF, Bowe JE, Kinsey-Jones JS, Brain SD, Lightman SL, O’Byrne KT. Differential role of corticotrophin-releasing factor receptors types 1 and 2 in stress-induced suppression of pulsatile luteinising hormone secretion in the female rat. J Neuroendocrinol 2006;18:602-10.

142. Kinsey-Jones JS, Li XF, Knox AM, Wilkinson ES, Zhu XL, Chaudhary AA, Milligan SR, Lightman SL, O’Byrne KT. Down-regulation of hypothalamic kisspeptin and its receptor, Kiss1r, mRNA expression is associated with stress-induced suppression of luteinising hormone secretion in the female rat. J Neuroendocrinol 2009;21:20-9.

143. Ebisui O, Fukata J, Tominaga T, Murakami N, Kobayashi H, Segawa H, Muro S, Naito Y, Nakai Y, Masui Y, et al. Roles of interleukin-1 alpha and -1 beta in endotoxin-induced suppression of plasma gonadotropin levels in rats. Endocrinology 1992;130:3307-13.

144. He D, Sato I, Kimura F, Akema T. Lipopolysaccharide inhibits luteinizing hormone release through interaction with opioid and excitatory amino acid inputs to gonadotropin-releasing hormone neurones in female rats: possible evidence for a common mechanism involved in infection and immobilization stress. J Neuroendocrinol 2003;15:559-63.

145. Xiao E, Xia-Zhang L, Ferin M. Inhibitory effects of endotoxin on LH secretion in the ovariectomized monkey are prevented by naloxone but not by an interleukin-1 receptor antagonist. Neuroimmunomodulation 2000;7:6-15.

146. Knox AM, Li XF, Kinsey-Jones JS, Wilkinson ES, Wu XQ, Cheng YS, Milligan SR, Lightman SL, O’Byrne KT. Neonatal lipopolysaccharide exposure delays puberty and alters hypothalamic Kiss1 and Kiss1r mRNA expression in the female rat. J Neuroendocrinol 2009;21:683-9.

147. Iwasa T, Matsuizaki T, Tungalagsuvd A, Munkhzaya M, Kawai T, Niki H, Kato T, Kuwahara A, Uemura H, Yasui T, Irahara M. Hypothalamic Kiss1 and RFRP gene expressions are changed by a high dose of lipopolysaccharide in female rats. Horm Behav 2014;66:309-16.

148. Chan YM, Butler JP, Pinnell NE, Pralong FP, Crowley WF Jr, Ren C, Chan KK, Seminara SB. Kisspeptin resets the hypothalamic GnRH clock in men. J Clin Endocrinol Metab 2011;96:E908-15.

149. George JT, Veldhuis JD, Roseweir AK, Newton CL, Facenda E, Millar RP, Anderson RA. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J Clin Endocrinol Metab 2011;96:E1228-36.

150. Jayasena CN, Nijher GM, Chaudhri OB, Murphy KG, Ranger A, Lim A, Patel D, Mehta A, Todd C, Ramachandran R, Salem V, Stamp GW, Donaldson M, Ghtei MA, Bloom SR, Dhillon WS. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. J Clin Endocrinol Metab 2009;94:4315-23.

151. Jayasena CN, Nijher GM, Abbara A, Murphy KG, Lim A, Patel D, Mehta A, Todd C, Donaldson M, Trew GH, Ghaete MA, Bloom SR, Dhillon WS. Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. Clin Pharmacol Ther 2010;88:840-7.

152. Jayasena CN, Connminos AN, Veldhuis JD, Misra S, Abbara A, Izzi-Engbeaya C, Donaldson M, Ghtei MA, Bloom SR, Dhillon WS. A single injection of kisspeptin-54 temporarily increases luteinizing hormone pulsatility in healthy women. Clin Endocrinol (Oxf) 2013;79:558-63.

153. Jayasena CN, Abbara A, Veldhuis JD, Connminos AN, Ratnasabapathy R, De Silva A, Nijher GM, Ganiry-Dada Z, Mehta A, Todd C, Ghtei MA, Bloom SR, Dhillon WS. Increasing LH pulsatility in women with hypothalamic amenorrhea using intravenous infusion of Kisspeptin-54. J Clin Endocrinol Metab 2014;99:E953-61.

154. Jayasena CN, Nijher GM, Connminos AN, Abbara A, Janniszewski A, Vaal ML, Sriskandarajah L, Murphy KG, Farzad Z, Ghtei MA, Bloom SR, Dhillon WS. The effects of kisspeptin-10 on reproductive hormone release show sexual dimorphism in humans. J Clin Endocrinol Metab
161. Anderson GM, Hoffman GE, Anselmo-Franci JA, Kisspeptin and Reproduction
162. Szawka RE, Ribeiro AB, Leite CM, Helena CV, Franci CR, 163. Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. Clin Endocrinol (Oxf) 2013;79:100-4.
164. George JT, Veldhuis JD, Tena-Sempere M, Millar RP, Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. Clin Endocrinol (Oxf) 2013;79:100-4.
165. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.
166. Seminara SB, Diptiero MJ, Ramaswamy S, Crowley WF Jr, Plant TM. Continuous human metastin 45-45 infusion desensitizes G protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male Rhesus monkey (Macaca mulatta): a finding with therapeutic implications. Endocrinology 2006;147:2122-6.
167. Baba T, Kang HS, Hosoe Y, Kharma B, Abiko K, Matsunaga M, Mandai M, Murphy SK, Konishi I. Menstrual cyclic change of metastin/GPR54 in endometrium. Med Mol Morphol. Epub 2014 Jun 8. DOI: http://dx.doi.org/10.1007/s00795-014-0081-0.
168. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.
169. Seminara SB, Diptiero MJ, Ramaswamy S, Crowley WF Jr, Plant TM. Continuous human metastin 45-45 infusion desensitizes G protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male Rhesus monkey (Macaca mulatta): a finding with therapeutic implications. Endocrinology 2006;147:2122-6.
170. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.
171. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.
172. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.
173. Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghaete MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074-82.