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Comprehensive Review on Kisspeptin and Its Role in Reproductive Disorders

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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.

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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 anestrus 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 IR in Turkish hamsters. They found increased kisspeptin IR in the AVPV in hamsters exposed to long day conditions, versus short day and long day-pinealectomised hamsters, suggesting a close relationship with melatonin in the regulation of seasonal reproduction [59].

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-

speptin, 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 κ-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 paracine 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 gonadotrophin 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 hypoestrogenic 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: ERα and ERα. Estrogen is known to exert its positive feedback via centrally located ERα to induce the LH surge [89,90]. However, GnRH neurons lack the ERα 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 ERα. 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 estogen-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]. Futhermore, 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]. Futhermore, 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 hypogonadtrophic 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 wil type controls. However, no difference in

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 ovariactomized 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 supressed e.g., hormone sensitive cancers.

Human studies investigated the effects of exogenous kisspeptin on LH secretion. In 2005 Dhillo 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 women in the follicular phase of the menstrual cycle. However, Chan et al. [155] observed LH responses 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.

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