REVIEW

Kisspeptin and fertility

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Abstract

The kisspeptins are a family of peptide hormones, which in recent years have been shown to play a critical role in the regulation of the hypothalamic–pituitary–gonadal axis, thus in turn influencing fertility and reproduction. This review examines the physiological role of kisspeptin and the kisspeptin receptor in the control of gonadotrophin and gonadal steroid hormone secretion and the implications of these findings with respect to fertility. In addition, the potential therapeutic use of kisspeptin in the treatment of reproductive disorders will be examined.

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Introduction

Infertility affects up to one in six couples in the United Kingdom (Human Fertilisation and Embryology Authority 2009, http://www.hfea.gov.uk/infertility-facts.html#1248). The currently available hormone-based treatments for infertility act through the manipulation of the hypothalamic–pituitary–gonadal (HPG) axis at the level of GnRH or below. Although effective, these treatments also have a significant failure rate as well as an associated morbidity. The discovery of kisspeptin in 1996 and the subsequent identification of the kisspeptin receptor (previously known as G-protein-coupled receptor 54, GPR54) have added a new critical dimension to our understanding of the physiology of the HPG axis, reproduction and fertility (Lee *et al.* 1996, Clements *et al.* 2001). Mice and humans lacking kisspeptin receptor expression show a phenotype of hypogonadotrophic hypogonadism and consequent infertility (de Roux *et al.* 2003, Seminara *et al.* 2003). However, kisspeptin receptor knockout mice have normal levels of hypothalamic GnRH expression (Seminara *et al.* 2003) and normal GnRH neuronal morphology (Messager *et al.* 2005). These findings have been pivotal in the emergence of kisspeptin signalling as critical regulator of normal fertility, and future work may lead to the development of therapeutic use of kisspeptin in the treatment of reproductive disorders.

Kisspeptin structure and distribution

The kisspeptins are a family of RF peptide hormones, so named as the arginine–phenylalanine residues are present at the amino terminal (Arg-Phe-NH2; Clements *et al.* 2001, Kotani *et al.* 2001). The kisspeptins are products of the *KISS1* gene derived from the plasma proteolytic cleavage of the 145-amino acid gene product, the suffix denoting the number of amino acids. All of the kisspeptin fragments have a C-terminal decapeptide that is critical for biological activity, and all of the kisspeptin forms show similar agonist activity for kisspeptin receptor (Clements *et al.* 2001, Kotani *et al.* 2001, Ohtaki *et al.* 2001).

Kisspeptin is found in both the peripheral and the central nervous system (CNS). In the periphery, kisspeptin has been identified in the testis, ovary, anterior pituitary gonadotrophs, pancreas and small intestine (Ohtaki *et al.* 2001, Richard *et al.* 2008, Gaytan *et al.* 2009). However, peripheral expression of kisspeptin is highest in the placenta with maternal plasma levels of kisspeptin in the third trimester of pregnancy rising to 7000-fold greater than in the non-pregnant state (Muir *et al.* 2001, Ohtaki *et al.* 2001, Horikoshi *et al.* 2003). It has been postulated that the function of kisspeptin production by the placenta may be to down-regulate the HPG axis during pregnancy. However, this is not in keeping with the finding that when kisspeptin-10 is centrally administered to pregnant rats, stimulation of the HPG axis is found to be preserved (Roa *et al.* 2006). Kisspeptin-10 inhibits trophoblast migration in human placental explants (Bilban *et al.* 2004). Plasma kisspeptin IR is elevated in patients with gestational trophoblastic neoplasia when compared with non-pregnant controls and falls during and after chemotherapy (Dhillo *et al.* 2006). Furthermore, levels of kisspeptin and kisspeptin receptor mRNA in placental tissue are increased in cases of gestational trophoblastic disease when compared with normal placental tissue (Janneau *et al.* 2002). Such observations have

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led to speculation that kisspeptin may act to regulate trophoblastic invasion of uterine tissue. However, patients with inactivating mutations of the kisspeptin receptor are able to undergo normal pregnancy after treatment with GnRH or gonadotrophin (Pallais *et al.* 2006). The function of placental kisspeptin signalling is therefore currently unknown; however, its presence may not be essential for placental function in humans.

Within the rodent CNS, both *Kiss1* mRNA and kisspeptin protein are particularly highly expressed within the hypothalamus in the arcuate nucleus (ARC), anteroventral periventricular nucleus (AVPV) and periventricular nucleus (Gottsch *et al.* 2004). In primates including humans, hypothalamic *KISS1* mRNA is predominantly found within the infundibular nucleus, which is the equivalent of the ARC in this order of mammals (Rometo *et al.* 2007).

The kisspeptin receptor

The kisspeptin receptor is a member of the rhodopsin family of seven transmembrane GPRs with structural similarities to the galanin receptor. However, it does not appear to bind galanin *in vitro* (Lee *et al.* 1999, Muir *et al.* 2001). Initially classified as an orphan receptor, the kisspeptin receptor (previously known as GPR54) was found to be the cognate receptor for kisspeptin 5 years after the hormone was first described (Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001).

Within the CNS, the kisspeptin receptor is found within the hypothalamus but is also widely expressed within both cortical and subcortical regions (Lee *et al.* 1999). In the periphery, it is notably expressed in the placenta and by pituitary gonadotrophs (Muir *et al.* 2001, Richard *et al.* 2008).

Kisspeptin stimulates the release of GnRH through action at the kisspeptin receptor, which in turn stimulates gonadotrophin release

The GnRH neurons of primates, rodents and sheep are found in close apposition with kisspeptin neurons (Silverman *et al.* 1977, Barry 1979, Rance *et al.* 1994, Clarkson & Herbison 2006). GnRH neurons express the kisspeptin receptor, and when kisspeptin is incubated with hypothalamic explants, it stimulates the release of GnRH. This effect is not observed in kisspeptin receptor knockout mice (*kiss1r*$^{-/-}$; d'Anglemont de Tassigny *et al.* 2008). The pivotal role of the kisspeptin receptor in kisspeptin-stimulated GnRH release is further demonstrated by the attenuation of the rise in plasma LH following kisspeptin administration in male mice and rats pre-treated with i.c.v. injections of a kisspeptin receptor (Roseweir *et al.* 2009).

I.c.v. administration of kisspeptin-52 to male rodents results in the expression of c-Fos (a well-established marker of neuronal activation) within the cell bodies of GnRH neurons. In addition, GnRH neurons show an increase in firing rate *in vitro* following kisspeptin treatment. This effect is attenuated by the application of a kisspeptin receptor antagonist (Irwig *et al.* 2004, Liu *et al.* 2008, Roseweir *et al.* 2009). Furthermore, kisspeptin treatment results in a dose- and time-dependent increase in *GnRH* mRNA levels in GnRH-secreting neuronal cell lines (Novaira *et al.* 2009).

Both i.c.v. and peripheral administration of kisspeptin result in a marked rise in plasma LH and to a lesser extent FSH in several mammalian species including rats, mice, sheep, monkeys and humans. This effect is abolished in *GPR54*$^{-/-}$ mice (Gottsch *et al.* 2004, Thompson *et al.* 2004, Dhillo *et al.* 2005, 2007, Messager *et al.* 2005, Seminara *et al.* 2006, Caraty *et al.* 2007). The effect of kisspeptin on gonadotrophin release is likely to be due to kisspeptin stimulation of GnRH release into the portal circulation, which in turn stimulates the release of LH and FSH from the gonadotrophs of the anterior pituitary gland. Evidence for this includes the abolition of the kisspeptin-induced gonadotrophin rise following pre-treatment with a GnRH antagonist.

These findings are consistent with the phenotype of hypogonadotrophic hypogonadism of *kiss1r*$^{-/-}$ and *kiss1*$^{-/-}$ null mice (de Roux *et al.* 2003, Seminara *et al.* 2003, d'Anglemont de Tassigny *et al.* 2007). These mice have normal levels of hypothalamic GnRH. As expected, kisspeptin administration to *Kiss1*$^{-/-}$ mice results in gonadotrophin release, but this effect is not observed in *Gpr54*$^{-/-}$ mice (de Roux *et al.* 2003, Seminara *et al.* 2003, Messager *et al.* 2005, d'Anglemont de Tassigny *et al.* 2007). Thus, evidence suggests that kisspeptin is the major ligand for the kisspeptin receptor.

Kisspeptin may also act directly on pituitary gonadotrophs to stimulate gonadotrophin release

Some lines of evidence point towards a direct effect of kisspeptin on the gonadotrophs of the anterior pituitary gland, stimulating the release of LH and FSH. Both the *Kiss1* and *Kiss1R* genes are expressed in pituitary gonadotrophs, and *in vitro* exposure of pituitary cells and tissue explants to kisspeptin results in the dose-dependent release of LH (Kotani *et al.* 2001, Navarro *et al.* 2005, Gutierrez-Pascual *et al.* 2007, Richard *et al.* 2008). Furthermore, in ovariectomised female rats, there is a fall in the expression of *Kiss1* mRNA within gonadotrophs, which can be prevented by the administration of oestradiol (E_2; Richard *et al.* 2008). These data suggest that circulating sex steroids may be required for pituitary *Kiss1* expression. This is consistent with the observation that circulating levels of E_2 modulate responsiveness of pituitary gonadotrophs to GnRH (Knobil *et al.* 1980).

Nevertheless, *kiss1r*$^{-/-}$ mice that show a phenotype of hypogonadotrophic hypogonadism have preserved gonadotroph function as evidenced by an appropriate response to exogenous GnRH (Seminara *et al.* 2003). In addition, i.v. administration of kisspeptin fails to stimulate LH release in

GnRH-replaced, ovariectomised, hypothalamic–pituitary-disconnected ewes. Furthermore, the pre-treatment of male monkeys with a GnRH antagonist results in the failure of administered kisspeptin to stimulate an LH rise (Plant *et al.* 2006, Smith *et al.* 2008).

The role of kisspeptin within the pituitary is currently unknown. *In vivo* and *ex vivo* evidences suggest a stimulatory role of kisspeptin within the anterior pituitary. However, pituitary kisspeptin signalling does not appear critical for the robust stimulation of gonadotrophin release observed following kisspeptin administration *in vivo*. Hence, kisspeptin signalling within the pituitary may have a regulatory role on gonadotrophin function, which is distinct to its better characterised role within the hypothalamus.

Kisspeptin plays a key role in mediating gonadal steroid feedback to the hypothalamus

Steroid hormones produced by the gonads feed back to the hypothalamus exerting a positive or a negative regulatory effect on GnRH production and release. The oestrogen receptors (ERs) are transcription factors, which exist as two isoforms, ERα and ERβ. These receptors bind to specific DNA sequences known as oestrogen-response elements found in the promoter region of oestrogen-responsive genes, resulting in the activation or suppression of gene transcription.

During most of the menstrual cycle, oestrogen suppresses gonadotrophin secretion, but at mid-cycle, the effect of oestrogen on the HPG axis changes to a potent positive feedback effect, leading to a surge in the plasma LH and ovulation. Increasing evidence suggests that hypothalamic kisspeptin signalling plays a critical role in the generation of the pre-ovulatory LH surge, which is necessary for normal fertility. Evidence for this comes from ovariectomised *GPR54* and *Kiss1* null mice treated with oestrogen and progesterone, which fail to mount an LH surge unlike their wild-type litter mates (Dungan *et al.* 2007, Clarkson *et al.* 2008). In addition, infusion of a monoclonal anti-rat kisspeptin antibody into the pre-optic area (POA), which contains GnRH cell bodies, leads to the complete blockade of the pre-ovulatory LH surge in oestrogen-treated ovariectomised female rats (Kinoshita *et al.* 2005). Lastly, continuous i.c.v. administration of a kisspeptin antagonist to female rats in the morning of oestrous until the afternoon of the following pro-oestrous prevents the pre-ovulatory LH surge (Pineda *et al.* 2010).

ERα has been shown to be critical for the positive feedback effect of oestrogen, which results in the female mid-cycle LH surge. Adult female rodents pre-administered with an ERα antagonist fail to ovulate or undergo a pre-ovulatory LH surge and have blunted LH response following administration of kisspeptin-10 (Roa *et al.* 2008). Furthermore, ovariectomised, oestrogen-replaced, neuron-specific *ERα* null mice are infertile. It is not known whether such mice have altered levels of *Kiss1* expression, but they fail to generate a pre-ovulatory LH peak despite having normal basal levels of LH (Wintermantel *et al.* 2006). Interestingly, the pre-ovulatory LH surge is preserved in *ERβ* knockouts (Wintermantel *et al.* 2006). GnRH neurons express ERβ but not ERα, which implies that the mid-cycle positive regulation of GnRH release by oestrogen is mediated by a separate population of ERα-expressing neurons with afferent input to GnRH neurons (Hrabovszky *et al.* 2000). Dual *in situ* hybridisation studies have revealed the co-expression of *Kiss1* and *ERα* mRNA in the AVPV and the ARC. In addition, work using neuronal viral retrograde tracing in mice has demonstrated ERα-expressing neurons in the AVPV and ARC with afferent input to GnRH neurons (Smith *et al.* 2005, Wintermantel *et al.* 2006). In female rats, *Kiss1* mRNA expression in the AVPV peaks during the evening of pro-oestrous but falls to a nadir in the ARC (Smith *et al.* 2006*b*). *Kiss1* neurons in the AVPV show high levels of c-Fos expression during the LH surge, while c-Fos expression at dioestrous is virtually absent (Smith *et al.* 2006*b*). Conversely, c-Fos is almost undetectable in the ARC at pro-oestrous (Smith *et al.* 2006*b*). Lastly, in female mice following ovariectomy, *Kiss1* expression in the AVPV falls but is increased following oestrogen replacement in these animals. Interestingly, the opposite pattern is observed in the ARC (Smith *et al.* 2005).

Taken altogether, these findings suggest that E_2 within ERα-expressing kisspeptin neurons in the AVPV positively regulates GnRH neurons culminating in the pre-ovulatory LH surge. However, ERα-expressing kisspeptin neurons in the ARC respond to oestrogen stimulation by inhibiting GnRH production and release (Fig. 1). This feed forward-feedback control on GnRH release by gonadal steroids is critical for normal fertility.

Kisspeptin neurons in the ARC co-express other neuropeptides, which regulate the HPG axis

Kisspeptin neurons in the ARC co-express the neuropeptides neurokinin B (NKB) and dynorphin, and this co-localisation is highly conserved in several mammalian species including humans (Goodman *et al.* 2007, Rance 2009, Hrabovszky *et al.* 2010). Dynorphin is an endogenous opioid peptide, which plays a role in the progesterone-mediated negative feedback control of GnRH release, while NKB is a member of the substance P-related tachykinin family (Goodman *et al.* 2004, Krajewski *et al.* 2005). The receptor for NKB is tachykinin neurokinin 3 receptor (NK3R), which is expressed on GnRH neurons (Todman *et al.* 2005).

Recently, it has been reported in humans that loss-of-function mutations in the gene encoding NKB (*TAC3*) or the gene encoding NK3R (*TAC3R*) result in normosmic hypogonadotrophic hypogonadism and pubertal failure. This phenotype is remarkably similar to rodent and human models of defective kisspeptin signalling (de Roux *et al.* 2003,

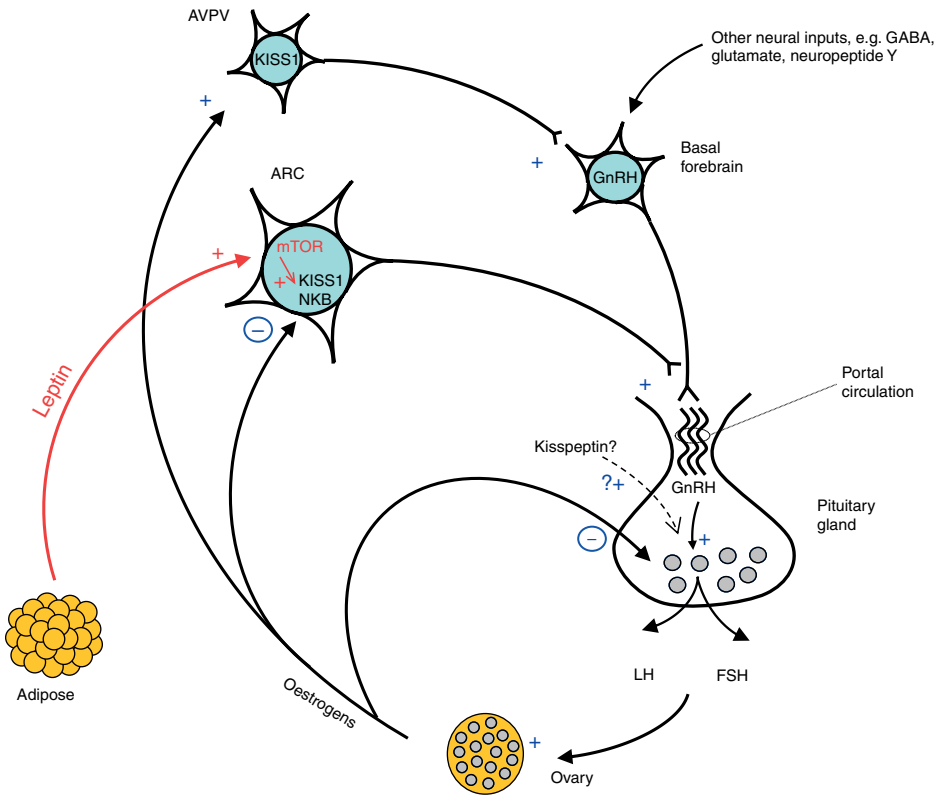

Figure 1 Diagram of kisspeptin signalling within the female central nervous system. KISS1-expressing neurons within the hypothalamic arcuate nucleus (ARC; equivalent to the infundibular nucleus in primates) are negatively regulated by oestrogen and inhibit GnRH release. KISS1-expressing neurons within the hypothalamic anteroventral periventricular nuclear (AVPV) are positively regulated by oestrogen and stimulate GnRH during the pre-ovulatory surge. Kisspeptin may also have a direct modulatory effect on pituitary LH and FSH release. KISS1 neurons in the ARC co-express neurokinin B (NKB), which stimulates LH and FSH release in a GnRH-dependent manner. Leptin signalling has a permissive effect on kisspeptin signalling within the ARC, which may be mediated by the mammalian target of rapamycin (mTOR; also known as mechanistic target of rapamycin, MTOR) pathway.

Seminara *et al.* 2003, d'Anglemont de Tassigny *et al.* 2007, Guran *et al.* 2009, Topaloglu *et al.* 2009).

Kisspeptin neurons in the ARC project to GnRH neurons. The association of NKB and kisspeptin within kisspeptin neurons may be suggestive of a synergy between the two neuropeptides in the regulation of the HPG axis. For example, it is known that the expression of both peptides is inhibited by E_2. In addition, in post-menopausal women, the distribution and morphology of the hypertrophied *KISS1*-positive neurons in the infundibular nucleus are similar to that of *NKB*-expressing cells (Rometo *et al.* 2007, Navarro *et al.* 2009).

Until recently, the effect of NKB on reproductive hormone release had been inconclusive with conflicting results from studies in sheep and rodents (Jayasena & Dhillo 2010). However, recently it has been reported that i.v. administration of NKB or an NKB agonist to male monkeys results in a potent stimulation of LH release, an effect that is abolished by pre-treatment with a GnRH antagonist (Ramaswamy *et al.* 2010). Interestingly, repetitive administration of NKB is not associated with a sustained pattern of LH release (Ramaswamy *et al.* 2010). Furthermore, monkeys remain responsive to kisspeptin injection despite losing responsiveness to NKB following repetitive administration. This suggests that NKB stimulates GnRH release in a kisspeptin-independent manner. It is not known whether animals may respond to NKB despite losing responsiveness to kisspeptin following chronic administration; such an observation would suggest NKB and kisspeptin to have parallel influences of GnRH release.

A role for kisspeptin signalling in pubertal development and seasonal reproductive activity

During puberty, the immature mammal develops adult physical and hormonal characteristics rendering it fertile and capable of reproduction. Juvenile *kiss1r*$^{-/-}$ and *kiss1*$^{-/-}$ mice and humans with inactivating kisspeptin receptor mutations fail to enter puberty (de Roux *et al.* 2003,

Seminara *et al.* 2003, d'Anglemont de Tassigny *et al.* 2007); these observations therefore suggest that kisspeptin signalling plays a critical role in the onset of puberty.

Further evidence for the importance of kisspeptin signalling in the onset of puberty comes from studies of immature female rats administered twice daily i.c.v. injections of kisspeptin from postnatal day 26 to day 31 (Navarro *et al.* 2004). This results in precocious vaginal opening, increased uterine weight and raised plasma LH and E_2 levels relative to vehicle-treated controls. These findings complement the report of central precocious puberty in an 8-year-old girl due to an activating mutation of KISS1R (Teles *et al.* 2008).

At postnatal day 10, kisspeptin-expressing neurons in the AVPV are not detectable in either male or female mice. However, these become apparent, lying in close apposition to GnRH neurons, from postnatal day 25, reaching adult levels by the onset of puberty at postnatal day 31 (Clarkson & Herbison 2006). In support of these findings, the expression of *KISS1* mRNA in the mediobasal hypothalamus (MBH) of agonadal male rhesus monkeys has been found to be significantly greater in the pubertal group than in the juvenile cohort (Shahab *et al.* 2005). Similarly, in female monkeys with intact gonads, a threefold increase in *KISS1* mRNA expression is observed in the MBH in early pubertal animals that in a juvenile group (Shahab *et al.* 2005).

Kisspeptin is also implicated in seasonal regulation of reproductive activity in seasonal breeders. In Syrian hamsters, hypothalamic *Kiss1* expression is high during long day conditions (associated with sexual activity) and reduced during short day conditions (associated with a quiescence of sexual activity); furthermore, administration of kisspeptin-10 to these animals restores testicular weight and testosterone release (Revel *et al.* 2006, 2007). Sheep display seasonal sexual activity during short day conditions. *KISS1* expression within the ARC is increased under short day conditions in ovariectomised ewes, but no seasonal change in *KISS1* expression is detected within the POA (Clarke *et al.* 2009).

These findings suggest that kisspeptin acts as an essential gatekeeper to the onset of puberty and consequent fertility in several mammalian species. Furthermore, kisspeptin may play a role in the seasonal regulation of reproductive activity in Syrian hamsters and sheep.

Kisspeptin provides a link between nutritional status and fertility

It has long been recognised that an intimate relationship exists between nutritional status and fertility. Leptin is a peptide hormone synthesised and secreted by adipocytes conveying information about body energy stores and nutritional status (Zhang *et al.* 1994, Pelleymounter *et al.* 1995). The level of circulating leptin is proportional to fat mass and falls in both mice and humans following weight loss (Maffei *et al.* 1995). The leptin-deficient *ob/ob* mouse is a well-established model of hypoleptinaemia (Zhang *et al.* 1994). These mice have delayed puberty and are infertile as a consequence of hypogonadotrophic hypogonadism. Interestingly, a similar phenotype of hypogonadotrophic hypogonadism is also present in humans with mutations of leptin or its receptor and in women with hypoleptinaemia as a consequence of low body weight (Coleman 1978, Clement *et al.* 1998, Farooqi *et al.* 1999, Welt *et al.* 2004). Leptin therapy in hypoleptinaemic mice and humans reverses these reproductive abnormalities. In addition, when leptin is administered to juvenile wild-type mice, this accelerates the onset of puberty (Chehab *et al.* 1996, 1997, Ahima *et al.* 1997, Farooqi *et al.* 1999, Welt *et al.* 2004).

The leptin receptor (Ob-Rb) is not expressed by GnRH neurons of the hypothalamus. However *Ob-Rb* mRNA is found in 40% of *Kiss1* mRNA-expressing cells of the ARC (Smith *et al.* 2006*a*). The expression of *Kiss1* mRNA in the ARC of *ob/ob* mice is reduced in comparison with wild-type mice. Interestingly, although kisspeptin mRNA in the ARC of *ob/ob* mice is significantly increased following leptin treatment, its expression is not restored to that of the wild-type controls (Smith *et al.* 2006*a*).

The expression of *Kiss1* mRNA has been shown to be influenced by nutritional status. In pre-pubertal rats, which have been food deprived for 72 h, the hypothalamic expression of *Kiss1* mRNA is markedly reduced (Castellano *et al.* 2005). In a model of chronic undernutrition in pre-pubertal rats, daily i.c.v. administration of kisspeptin from postnatal day 30 to 37 restores the delayed vaginal opening of these animals and increases the suppressed levels of plasma LH, FSH and E_2 (Castellano *et al.* 2005).

The ARC contains discrete subpopulations of first-order leptin-responsive neurons. One subpopulation expresses the orexigenic peptide neuropeptide Y (NPY), while another expresses the anorectic peptide α-melanocyte-stimulating hormone (α-MSH), which is derived from pro-opiomelanocortin (POMC; Cheung *et al.* 1997, Broberger *et al.* 1998). Using double-label fluorescent immunohistochemistry, kisspeptin fibres in ewes have been shown to be in close apposition with ARC NPY and POMC neurons (Backholer *et al.* 2010). I.c.v. administration of an α-MSH-like agonist to ewes increases *KISS1* mRNA in the POA and results in a rise in plasma LH (Backholer *et al.* 2009). Conversely, the orexigenic peptide melanin-concentrating hormone, which is highly expressed in the lateral hypothalamus, inhibits the stimulatory effects of kisspeptin on GnRH neurons (Wu *et al.* 2009).

Mammalian target of rapamycin protein (mTOR; also known as mechanistic target of rapamycin, MTOR) is another regulator of energy homeostasis, which has been shown to influence kisspeptin signalling. mTOR is a ubiquitously expressed serine-threonine protein kinase, which plays a vital role in the regulation of cell growth and differentiation (Schmelzle & Hall 2000). When nutrient availability is low, mTOR activity falls thus inhibiting the high-energy demand cell cycle. Interestingly, i.c.v. administration of leucine (which stimulates mTOR signalling) leads to an increase in plasma LH in female peri-pubertal rats

(Cota *et al.* 2006, Roa *et al.* 2009). Conversely, when mTOR activation is blocked (by rapamycin), the expression of *Kiss1* mRNA in the ARC becomes almost undetectable, and plasma levels of LH fall (Roa *et al.* 2009).

Reproduction is a highly energy demanding process, and during food deprivation the HPG axis is down-regulated in order to conserve energy. In summary, the findings presented above demonstrate a key role for hypothalamic kisspeptin as a link between nutritional status and fertility.

Could kisspeptin offer a novel therapy for the treatment of fertility disorders?

As our understanding of the biology of the kisspeptin signalling system grows, it is becoming increasingly tempting to speculate on the possible therapeutic use of kisspeptin in the treatment of fertility disorders. Infertility affects up to one in six couples in the United Kingdom, and although effective, both the hormonal and surgical therapies, which are currently employed are not without significant side effects and failure rates (National Institute for Health and Clinical Excellence 2004, Human Fertilisation and Embryology Authority 2009, http://www.hfea.gov.uk/infertility-facts.html#1248).

When administered to healthy male subjects, i.v. infusion of kisspeptin results in a significant increase in plasma LH, FSH and testosterone (Dhillo *et al.* 2005). S.c. injection of kisspeptin to healthy pre-menopausal female subjects elicits a marked rise in LH, which is most pronounced in the pre-ovulatory phase of the menstrual cycle (Dhillo *et al.* 2007). This finding sits well with the animal studies reviewed above, which suggest an essential role for kisspeptin in the generation of the LH surge.

Kisspeptin has been administered to humans by s.c. and i.v. injection without any observed adverse effects (Dhillo *et al.* 2005, 2007). In particular, kisspeptin has not been shown to cause changes in heart rate or blood pressure, which is important in view of the vasoconstrictor properties of kisspeptin that have been demonstrated *in vitro* (Mead *et al.* 2007, Nijher *et al.* 2010).

When kisspeptin is administered twice daily s.c. injection to infertile women with functional hypothalamic amenorrhoea (HA) due to low body weight, it effectively stimulates a rise in plasma gonadotrophins (Jayasena *et al.* 2009). This effect is most marked following the first injection of kisspeptin and is significantly diminished after 2 weeks of treatment. However, biweekly administration of kisspeptin results in a sustained gonadotrophin response to kisspeptin (Jayasena *et al.* 2010). Furthermore, it has been shown that in women with HA, the LH response to injected kisspeptin is fourfold greater than that of healthy female subjects studied in the follicular phase (Jayasena *et al.* 2009). This may be due to an enhanced responsiveness to kisspeptin in women with HA or increased pituitary sensitivity to the effects of GnRH.

Gonadotrophin injections provide the current mainstay of infertility therapy (Elchalal & Schenker 1997). It is therefore interesting to consider if kisspeptin-based therapies would offer any potential advantages over existing therapies. Kisspeptin acts by stimulating endogenous hypothalamic GnRH release, which in turn triggers endogenous pituitary gonadotrophin release (Irwig *et al.* 2004, Thompson *et al.* 2004, Shahab *et al.* 2005). Kisspeptin therapy might therefore stimulate a more natural pattern of reproductive hormone release than existing therapies. Furthermore, the stimulation of endogenous gonadotrophin release by kisspeptin may be predicted to confer a lower risk of ovarian hyperstimulation syndrome associated with exogenous gonadotrophin injections (Elchalal & Schenker 1997). More data are needed to test if such potential benefits would exist over current therapies for infertility.

Concluding remarks

Over the past decade, our increasing insight into the biology of the kisspeptin pathway has significantly added to our understanding of the physiology and pathophysiology of the HPG axis. Kisspeptin has now been safely and successfully used in both healthy and infertile human subjects, and it is possible that in the future the manipulation of kisspeptin signalling may be used in the treatment of reproductive disorders.

Declaration of interest

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

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