The GPR54-Kisspeptin complex in reproductive biology: neuroendocrine significance and implications for ovulation induction and contraception

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REVIEW

Abstract

KISS1 encodes the kisspeptin (KP) family of peptides which were originally characterised as potent antimetastatic agents in breast cancer and malignant melanoma cells. One member of this family of arginine-phenylalanine amide peptides, KP-54, was subsequently identified as the natural ligand for the G-protein coupled receptor-54 (GPR54). In addition to its importance as a metastatic suppressor, KP has been found to play a major neuroregulatory role in governing endogenous gonadotropin release by its modulation of the hypothalamic-pituitary-gonadal (HPG) axis. In humans, KISS1 mRNA has been localised to the hypothalamic anteroventral periventricular nucleus and arcuate nucleus. Although GPR54 is expressed in human pituitary cells, it is not presently known if gonadotrope cells themselves are targets for significant KP activity. It was recently shown that full disruption of the KP/GPR54 complex resulted in hypogonadotropic hypogonadism. Indeed, evidence now suggests that KP/GPR54 signalling during gestation is necessary for sexual differentiation and implicates activation of the KP/GPR54 complex as the single most important upstream event regulating GnRH release. Several compelling studies have placed KP as the leading candidate molecule responsible for initiating puberty, making this receptor-ligand complex of fundamental importance to the neuroendocrinology of reproduction. Here, we discuss key KP/GPR54 discovery events and present an evolution of KP biology in the context of recent animal and human experimental work. With evidence pointing to proper KP/GPR54 signalling as the principal trigger for activation of GnRH neurons and subsequent ovulation, elucidation of how this pathway is modulated is likely to bring novel pharmacologic strategies for fertility treatment (and contraception) within reach. Because the physiological significance KP is now acknowledged to extend well beyond cancer biology (and may also contribute to the pathophysiology of pre-eclampsia), KP represents an exciting research theme in human reproductive biology and neuroendocrinology.

INTRODUCTION

In 2003, a discovery highly significant for neuroendocrinologists and reproductive biologists was reported by two independent laboratories nearly simultaneously. Investigating patients with abnormally delayed sexual maturation, research teams in Boston [Seminara *et al*, 2003] and Paris [de Roux *et al*, 2003] described the same (but previously unrecognised) genetic defect resulting in elimination of GnRH release and hypothalamic hypogonadism. The researchers focused on a single loss-of-function mutation in the human *KISS1* receptor gene present in some members of a consanguineous kindred, and when knock-out (null) mutations affecting the murine *KISS1* receptor gene homologue were produced with an identical physiologic result, the causative role of *KISS1* in orchestrating GnRH release was confirmed. Findings from these experiments moved the kisspeptins (KP) to the front of the line among factors considered responsible for initiating puberty and regulating reproductive function in general. As the central neurological process triggering puberty had long been elusive, the arrival of the KPs was welcome and appeared to supply a critical missing answer to this important unresolved question. Here we review selected events leading to the discovery of the KP ligand, its receptor, and manifestations of specific clinical conditions now attributed to their defects. Correction of such impairments is likely to be a development goal in drug discovery, also discussed here.

KISS-1, KISSPEPTIN AND GPR54

KISS1 was first recognised as a metastasis suppressor gene in melanomas and breast cancers [Lee *et al,* 1996]. The observation that malignant melanoma metastasis was suppressed by *KISS1* with no impact on tumour formation suggested the presence of a metastasis suppressive factor [Welch *et al,* 1994], and subtractive hybridization was utilised to map *KISS1* to chromosome 1q32 [Lee *et al*, 1996]. This metastasis suppressor activity of *KISS1* was subsequently confirmed in ovarian, melanoma, and breast cancer tissue [Lee *et al*, 1996; Lee and Welch, 1997a,1997b; Martin *et al,* 2005]. Interestingly, attenuated *KISS1* expression has emerged as a possible marker for poor prognosis in several types of cancer including bladder, thyroid, gastric, oesophageal, and hepatocellular carcinoma [Nash and Welch, 2006]. KPs have also been shown to have a potent vasoconstrictor action [Mead *et al*, 2007] and can play a down-regulatory role with respect to matrix metalloproteinases [Hesling *et al*, 2004]. Although further research seeks to determine how these properties might specifically mediate KPs metastasis suppression actions, parallel investigations are exploring the emerging and substantial contributions of the KPs to reproductive physiology [Jayasena *et al*, 2008].

The gene for the KP receptor (*KISS1R*) was identified over a decade ago [Lee *et al*, 1996] but the receptor could not be immediately paired to a natural ligand. While other non-uniform nomenclature including OT7T175 and AXOR12 was occasionally used for this orphan receptor, its expression was later confirmed in the hypothalamus, preoptic area, medulla and amygdala [Lee *et al*, 1999; Kotani *et al*, 2001; Muir *et al,* 2001]. Since the gene coding for GPR54 (in humans) was found to have sequence homology with the gene coding for galanin receptor 2 [Lee *et al*, 1999], it was characterised as a galanin-related orphan receptor (GPR54). Interestingly, the gene responsible for this orphan was also termed "Harry Potter" when its function was first altered in a murine model [Seminara *et al*, 2003].

KISS1 is the gene encoding the ligand, a 145-amino acid peptide which is cleaved to form biologically active fragments of varying length. To date, the full-length KP protein has not been detected intact as a secretory product [Nash *et al*, 2007]. Instead, the truncated fragments of KP have been derived from human placenta cells and have been termed metastin/kisspeptin-54 (54 amino acids), kisspeptin-14 (14 amino acids) and kisspeptin-13 (13 amino acids). These KP fragments share an identical 10 amino acid C-terminus peptide sequence [Kotani *et al,* 2001], which appears to be the functional component. This critical 10 amino acid portion of the kisspeptin ligand is highly conserved across species, and, when kept intact, there appears to be no difference in KP receptor binding at GPR54 irrespective of the total length of the peptide [Ohtaki *et al*, 2001].

RECENT EXPERIMENTAL FINDINGS

Robust evidence now exists to demonstrate that KP is intimately involved with key reproductive events. For example, KP increases GnRH neuron excitability [Han *et al,* 2005] and mouse studies have shown an increase in circulating gonadotropin concentrations following injection of KP into cerebral ventricles [Gottsch *et al*, 2004]. This stimulatory effect was extended to peripheral administration in sheep [Messager *et al*, 2005], rat [Matsui *et al*, 2004; Navarro *et al,* 2005], and pig [Lents and Barb, 2007]. To determine how KP elicited this gonadotropin response, KP was placed proximal to GnRH cell bodies situated in the medial preoptic area, and LH release was recorded [Patterson *et al,* 2006]. Interestingly, this stimulatory effect of KP was interrupted upon administration of GnRH antagonist [Gottsch *et al,* 2004; Irwig *et al*, 2004]. In the rat, injection of KP antiserum also obliterates the LH surge [Kinoshita *et al,* 2005]. An interesting experiment on humans (males) involved subcutaneous infusion of KP54, and reported a greater than two-fold increase in mean plasma LH

and an 18% increase in FSH, compared to saline controls [Dhillo *et al,* 2005].

As the connection between KP and GnRH became more definitive, additional experiments were undertaken to localise KP to specific neural tissues. Immunolabelling studies have found KP-reactive fibres near GnRH cell nuclei [Kinoshita *et al,* 2005; Clarkson and Herbison, 2006], and sheep studies have mapped *KISS1* mRNA to the arcuate nucleus and preoptic area [Smith *et al*, 2007a]. Murine research has identified *KISS1* mRNA in the periventricular nucleus, anteroventral periventricular nucleus, arcuate nucleus, and medial amygdala [Gottsch *et al*, 2004]. A paucity of KP-reactive cells has been identified in the dorsomedial hypothalamic nucleus where *KISS1* mRNA has not yet been identified, although this may be due to antisera cross-reactivity with other RF-amide substrates [Caraty and Franceschini, 2008].

Sex steroids probably play a substantial role in modulating mammalian *KISS1* mRNA expression in many of these sites. For example, mRNA expression is upregulated in the arcuate nucleus following oophorectomy although this effect is reversed by administration of exogenous oestradiol [Smith *et al*, 2005; Maeda *et al*, 2007]. Additionally, murine *Kiss1* mRNA expression has been found to fluctuate as a function of the oestrus cycle [Kinoshita *et al*, 2005], while rat and mouse studies have demonstrated the presence of oestradiol receptor alpha (ERα) on membrane of KP cells [Smith *et al,* 2005]. Ovine research has also identified ERα on KP cells of the arcuate nucleus [Franceschini *et al*, 2006].

In rodents, differential effects of sex steroids seem to be mediated in various brain regions [Smith *et al*, 2005; Adachi *et al*, 2007]. Female rats can undergo ovulation induction after a single subcutaneous injection of KP [Matsui *et al,* 2004] following pre-treatment with gonadotropin, and AVPV neurons expressing *Kiss1* in female rodents likely channel oestrogen signals to GnRH neurons to help evoke this preovulatory LH surge [Clarkson *et al*, 2008; Kauffman, 2008]. Additionally, the GnRH/LH surge in some species is influenced by an endogenous circadian rhythm which brings in additional signalling from the suprachiasmatic nucleus to modify KP release [Gu and Simerly, 1997]. Although GPR54 has been localised to pituitary and the in vitro stimulatory effect of KP on gonadotrope cells has been experimentally confirmed, GnRH signalling in these cells is still necessary for KP to cause gonadotropin release in vivo [Smith *et al*, 2007b].

KP IN SEXUAL DEVELOPMENT

It has long been speculated that differences in reproductive behaviour are a manifestation of corresponding sex-based differences in brain circuitry [Cooke *et al*, 1998; Simerly, 2002; Morris *et al*, 2004]. Such programming of brain differentiation based on a male or female template is accomplished by the ambient hormonal milieu present during the perinatal period [Simerly, 2002], the sequence and duration of which is probably species specific. Indeed, neuroanatomical studies in animals has shown the medial preoptic nucleus is more densely populated with neurons in males than in females, yet this pattern is reversed in the AVPV [Cooke *et al*, 1998; Simerly, 2002]. The actual functional significance of these dimorphic phenotypes, however, has been difficult to define [Shah *et al,* 2004]. Current research suggests that KP plays a pivotal role in orchestrating these sex-related differences in brain morphology and could explain why females (but not males) are able to produce a LH surge.

Specifically, *Kiss1* mRNA expressing neurons are found in significantly greater numbers in the AVPV of the adult rat compared to males [Kauffman *et al,* 2007] and these differences in *Kiss1* expression are maintained in adults irrespective of subsequent treatment with sex steroids [Adachi *et al,* 2007; Kauffman *et al,* 2007]. The situation is quite different when sex hormones are administered during the perinatal period, however. For example, a single injection of androgen given to female rodents (on the day of birth) results in a substantially attenuated population of *Kiss1* neurons, similar to that observed in males [Kauffman *et al*, 2007]. These observations strongly suggest KP deployment in the AVPV is determined by perinatal sex steroids, resulting in important adult sexual dimorphism with respect to *Kiss1* neuron expression in the AVPV. Recent animal experimentation has suggested that the presence or absence of these AVPV neurons expressing *Kiss1* potential corresponds to the functional potential to mount a LH surge [Kauffman, 2008].

ROLE OF KP IN JUVENILE-PUBERTY TRANSITION

The onset of normal puberty in higher primates (including humans) does not appear for some years after birth due to central neural suppression of GnRH release. This inhibition is not a result of intrinsic gonadal, pituitary or hypothalamic arrest, but rather derives from an upstream process causing all distal elements of the cascade to await in dormancy [Plant *et al,* 1989]. Indeed, during the period immediately following birth GnRH levels show considerable pulsatility that is soon interrupted by a prolonged intermission during the juvenile phase. Resumed GnRH release at the physiologic

transition from the juvenile to puberty stage of development is therefore the second of two essential postnatal shifts responsible for the gonadal activation characteristic of normal puberty. This transition is preceded by the infant-to-juvenile "adjustment" resulting in marked attenuation of GnRH pulsatility; this puts reproductive development transiently on hold during the juvenile years.

Dampening of GnRH pulsatility during infancy is probably influenced by KP, a hypothesis supported by the observation that peripheral gonadotropins were undetectable in a two month old boy who had a loss of function mutation involving the KP receptor [Semple *et al*, 2005]. Additionally, pubertal and postpubertal patients with disruption of the KP receptor have been found to have hypogonadotropic hypogonadism [Seminara *et al*, 2003]. Accordingly, if KP input to the hypothalamic GnRH centre is blocked due to genetic error, then the potential for high GnRH pulsatility (at any developmental phase) is truncated and sexual infantilism results. Although further studies are planned, these data already suggest an important role for KP in the multimodal signalling required to restore pulsatile GnRH release in puberty.

This complement of other centrally-acting factors known to influence gonadotropin release includes neuropeptide Y (NPY), substance P, leptin and glutamate, each of which has been carefully investigated with respect to reproductive function. For example, the glutamate derivative D-cycloserine, when administered orally causes relative increases in plasma LH compared to saline controls in male volunteers [van Berckel *et al*, 1998]. Leptin, in contrast, appears more permissive than overtly stimulatory to the hypothalamic-pituitary-gonadal axis, as prolonged fasting (*i.e*., 72h) disrupts gonadotropin release in healthy females and males. While such fasting is accompanied by sharply reduced circulating leptin levels, infusion of replacement (recombinant) leptin reverses this downregulation and restores gonadotropin release to normal [Chan *et al,* 2006]. Appetite is stimulated by NPY, another hypothalamic neurotransmitter with apparently permissive input on gonadotropin regulation. Experimental boluses of NPY have no effect on ambient LH levels in males, but when the same NPY dose is given with GnRH, this combination greatly amplifies the LH response compared to that expected when GnRH is given alone [Watanobe *et al*, 1994]. KP is perhaps most similar to substance P in terms of its physiologic effect, as substance P infusion in males elicits a profound (nearly two-fold) rise over baseline in plasma LH with no measurable effect on FSH [Coiro *et al*, 1992]. As the characterisation of KP is brought more clearly into focus, better understanding of interactions among these (and other) neurotransmitters is also likely to be achieved.

DISCUSSION

Some 30 years after GnRH was established as the regulatory trigger of pituitary gonadotropin release, work by numerous investigators has finally succeeded in finding the upstream agonist responsible for hypothalamic GnRH flow. KP is probably involved in all phases of reproductive life. It is now recognised as a potent stimulator of GnRH release, with KP-releasing cells mainly situated in the preoptic area and arcuate nucleus. As the most potent GnRH secretagogue yet discovered, KP derived drug development will depend on pharmacologic modelling and dose determination studies. Animal assays suggest only trace amounts of KP may be necessary to achieve a meaningful physiologic response, since the potency to release LH by KP is at least an order of magnitude less than that required for GnRH release [Thompson *et al,* 2004]. Indeed, as most GnRH neurons express KP receptors, when exposed to KP at concentrations even in the femptomole range the peptide can initiate a powerful GnRH and LH response [Irwig *et al,* 2004; Messager *et al*, 2005].

Investigations of KP biology have substantially advanced the understanding of initiation of puberty and regulation of gonadotropin secretion throughout the reproductive cycle. The fact that animal experimentation has shown continuous KP administration causes LH surges suggests this paradigm may eventually find application in the clinical management of ovarian hyperstimulation syndrome. Yet observations in animal models have also framed important questions for the future. For example, how does KP rescue gonadotropin secretion in diabetic rats [Castellano *et al*, 2006; Hauge-Evans *et al,* 2006; Silvestre *et al,* 2008]? Given the finding that KP modulates adipocyte metabolism [Brown *et al*, 2008], what is the relationship between leptin and KP? Does ghrelin play an antagonist role (opposing KP) in the reproductive regulatory equation? [Martini *et al,* 2006]. As KP stimulation on gonadotropin release is maximal at the preovulatory phase [Dhillo *et al*, 2007], might this signalling be harnessed to achieve an enhanced follicular recruitment in ovulation induction for IVF? And since a mutation causing chronic agonist signalling at the KP receptor has been found to result in precocious puberty in humans [Teles *et al*, 2008], might pharmacologic modification here represent another way to manage this disease state?

While early KP research began with a focus on its metastasis suppressor functions, KP has subsequently emerged as the principal switch regulating the hypothalamic-pituitary-gonadal axis. The questions posed here place KP at the centre of an exciting future in our understanding of reproductive biology. The likely role of KP as an integrator for peripheral signalling such as nutritional status and sex steroids in the context of overall GnRH release assures continued interest in this area of research.

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