Kisspeptins in human reproduction—future therapeutic potential

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Abstract

Objective Kisspeptins (Kps), were first found to regulate the hypothalamopituitary-gonadal axis (HPG) axis in 2003, when two groups demonstrated that mutations of GPR54 causes idiopathic hypogonadotropic hypogonadism (IHH) characterized by delayed puberty. Objective of this review is to highlight both animal and human discoveries in KISS1/GPR54 system in last decade and extrapolate the therapeutic potential in humans from till date human studies.

Design A systematic review of international scientific literature by a search of PUBMED and the authors files was done for Kp in reproduction, metabolic control & signal transduction.

Setting None

Patient(s): In human studies—normal subjects patients with HH, or HA.

Capsule Kisspeptins, Upstreams modulators, influence Hypothalamic-Pituitary-Gonadal (HPG) axis and thus brain sexual maturation, puberty initiation as well as positive and negative feedback control on GnRH/LH release and integrate metabolic inputs with regards to fertility desirable or not nutritionally.

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Main outcome measures: Effects of Kp on puberty, brain sexual maturation, regulation of GnRH secretion, metabolic control of GnRH Neurons (N).

Results Kps/GPR54 are critical for brain sexual maturation, puberty and regulation of reproduction. Kps have been implicated in mediating signals to GnRH N—positive and negative feedback, metabolic input. Ability of Kp neurons to coordinate signals impinging on the HPG axis makes it one of most important regulators of reproductive axis since GnRH N's lack many receptors, with Kp neurons serving as upstream modulators.

Conclusions Kps have proven as pivotal regulators of the reproduction, with the ability to integrate signals from both internal and external sources. Knowledge about signaling mechanisms involved in Kp stimulation of GnRH and with human studies has made it possible that therapeutically available Kp agonists/antagonists may be used for treatment of delayed puberty/HH, Hypothalamic amenorrhea and in prevention of spread of malignant ovarian/gonadal malignancies along with uses in some eating disorders.

Keywords Kisspeptins · KISS1 receptor · Idiopathic hypogonadotropic hypogonadism · Puberty initiation · Sexual maturation control · Negative feedback control · Positive feedback control · Metabolic control of reproduction

Introduction

The incidence of infertility is on the increase globally being 1 in 6 couples in Europe. Although the basic components and some of the major regulatory elements of the Hypothalamic-Pituitary-Gonadal (HPG) axis have been identified, and well characterized in last decade, there is unanimous appreciation of the substantial expansion of our understanding of the neurochemical mechanisms

responsible for the control of gonadotropin secretion in mammalian and nonmammalian species in recent years. Animal studies are so important because GnRH neurons are scanty and only in the last decade in 1999–2000 promoter driven reporter genes were applied to the identification of GnRH neurons e.g. the green fluorescent protein (GFP) tagged GnRH neurons in rats were made available by Spergel/Suter and direct neuro electrophysiological studies were possible but till date no such model is available in primates [1, 2] to study GnRH neurophysiology.

The currently available hormone based treatments for infertility act through the manipulation of HPG axis at the level of GnRH neurons or below. Although effective, these treatments have a significant failure rate as well as associated morbidity. Our knowledge of the neuronal circuits and signals as well as of the hormonal factors responsible for the dynamic regulation of GnRH secretion at the hypothalamus has grown significantly recently

The discovery of kisspeptins in 1996 by Lee et al. [3] and the subsequent discovery of G Protein Coupled Receptor 54- (GPR54) witnessed indispensable pieces in the hierarchy of neuroendocrine factors governing reproductive function. Kisspeptins (Kp), a series of peptides encoded by the Kiss 1 gene are the endogenous ligands of GPR54. As it was discovered in Hershey it was decided to name the gene after Hershey's famous chocolate kisses. The KISS1 gene is located on human chromosome 1q32 and encodes a 145 amino acids (aa) precursor peptide [4] which by a number of proteolytic processes gives rise to an arrays of length Kp54, 14, 13, 10. Collectively these N terminal truncated peptides known as Kp's belong to a large family of peptides known as RFamide which all share a common Arg-Phe-NH₂ motif in their C terminus. The carboxy terminal amidated aa sequence of all Kps is sufficient for them to be able to bind to its receptor efficiently [5, 6]. After its initial discovery, the interest in physiological functions was rejuvenated after the simultaneous report of two cases of isolated forms of hypogonadotropic hypogonadism (IHH) resulting from inactivating mutations of GPR54 gene in 2003 [7, 8]. This finding unveiled previously unsuspected reproductive dimensions of this signaling system as a key player in the regulation of gonadotropic axis. Further support for these essential reproductive functions came from demonstration that mice engineered to lack GPR54 or kiss1 gene were a phenocopy of affected humans [9] thus demonstrating conserved role of Kp's in control of HPG axis in mammals.

Recently Gottsch et al. [10] proposed on the basis of guidelines of Human Genome Organization Gene Nomenclature Committee that the terms KISS1 and Kiss1 be used to name the primate and nonprimate genes respectively whereas their peptide products are termed kisspeptins (Kp) with a numeric expression indicating the number of aa residues.

Neuroanatomy of Kiss1 neurons as key stimulatory inputs to GnRH neurons

To clarify excitatory action of Kp's on GnRH neurons a number of studies have been conducted in mammals to map the neuroanatomical location of kiss 1 expressing neurons in specific hypothalamic areas and their eventual projections to GnRH neurons. These features have been well characterized in rodents where the initial *in situ* hybridization (ISH) analysis performed in 2005 demonstrated two main populations of Kiss1 neurons located in the arcuate nucleus (Arc) and anteroventroperiventricular area (AVPV) [11, 12]. Later studies using mostly immunohistochemistry (IHC), documented that such rostral Kp expression extend beyond the AVPV, appearing as a continuum that includes adjacent areas such as preoptic periventricular nucleus—a region also known as RP3V (rostral periventricular area of the 3rd ventricle) [13, 14].

In addition detailed neuroanatomical mapping of Kp fibers and their relation with GnRH neurons have been conducted in different mammalian species—see review [15]. The most consistent Kp neuronal population located in Arc (infundibular nucleus in humans) identified by ICC/ISH in humans, monkeys, sheep, rats, mice, hamsters etc. (Table 1). In rodents, rostrocaudal distribution/whereas in sheep, primates, goats, horses Kp's mainly located in middle and caudal level of [15] Arc nucleus.

Development of Kiss1 neuronal population in the Arc and AVPV: role in brain sex differentiation

One distinctive feature of Kiss1 neuronal populations is their sexual dimorphism which in rodents is specially apparent in the AVPV with female having more Kiss1 neurons as adults than males [16, 32]. In addition striking developmental changes in the numbers of Kiss1 neurons at the two prominent hypothalamic sites have been well characterized in mice and rats [13, 34]. These findings have paved the way for the analysis of the underlying mechanisms for such development patterns and their functional relevance in terms of timing of puberty and later manifestations of sexually dimorphic traits of the HPG axis, such as the preovulatory surge of gonadotropins which is distinctive of the female.

Thus exposure of female rats to high levels of testosterone during this period, a model that mimics the phenomenon whereby early androgenic input deprives the male brain of the neuronal setting needed to respond to oestrogenic positive feedback, induces the masculinization of the Kiss1 neuronal population of the AVPV [32]. This suggests that the increase in testosterone that occurs in males at this stage of development is responsible for the virtual absence of Kiss1 neurons in this rostral area. However mechanism of action of testosterone is also through conversion to estradiol

Table 1 Distribution of Kisspeptin/Kiss1 cells in the mammalian nervous system

Species	Preoptic region					Medial			ISH reference	ICC reference
	ARC	POA	AVPV	ME		DMH	VMH	AMG		
Human	+++	+							Rometo 2007 [17]	Hrabovszky 2010 [18]
Monkey	+++	++	+						Rometo 2007 [17], Shahab 2007 [19], Smith 2010 [20]	Ramaswamy 2008 [21], Ramaswamy 2010 [22]
Sheep	+++	++				+	+		Estrada 2006 [23], Smith 2007 [24], Smith 2008 [25], Smith 2009 [26]	Cheng 2010 [32], Franceschini 2006 [28], Goodman 2007 [29], Smith 2008 [25], Smith 2009 [26]
Rat	+++								Adachi 2007 [30], Irwig 2004 [31], Kauffman 2007 [32], Smith 2006 [33], Takasake 2009 [34]	Destroziers 2010 [35], Kinoshita 2005 [36], Takasake 2009 [34]
Mouse	++	+				+		+	Gottsch 2004 [37], Han 2005 [38], Kauffman 2007 [32], Smith 2005a [11], Smith 2005b [12]	Clarkson & Herbison 2006 [13], Clarkson 2009 [16]
Hamster	++								Ansel 2010 [39]	Grievies 2007 [40]
Goat	+++									Onkara 2009 [41], Wakabayashi 2010 [42]
Horse	+++							+		Decourt 2008 [43], Magee 2009 [44]

+++large (50–150), ++moderate (15–50), +few (<15 or nil reported)

in males for sexual maturation [45] and the development of sexually dimorphic nucleus of POA requires PGE2 albeit not direct sex steroid action [46]. On the other hand excess of oestrogenic actions on developing brain appear to persistently impair later expression of Kiss1 gene at the hypothalamus [47, 48]. Altogether, the above data illustrate the sensitivity of developing Kiss1 system to the organizing effects of sex steroids during critical window of development whose disruption is likely to induce durable effects on reproductive maturation and function later in life. Recent studies suggest strongly that Arc population of Kiss1 neurons indeed, is likely to be larger in female rodents [49] in keeping with data from other species like sheep [27], and human [18]. On the other hand the intimate mechanisms whereby sex steroids and other eventual modulators, shape the organization and development of the different populations of Kiss1 neurons at the hypothalamus are largely unknown. Nevertheless, efforts in this front have been initiated in by the analysis of the potential contribution of Bax mediated apoptosis [50], TRAF [51]. The suppression in males is the result of higher expression of an TNF α associated protein called TRIP (TNF α -receptor associated-associated factor2 inhibiting protein) which inhibits both the TNF α -NF-Kb survival pathway and the antiapoptotic protein, bcl-2 [52]. In the generation of sex dependent differences and the overall number of Kiss1 neurons of specific hypothalamic sites [53], a study that is likely to expand upon potential contribution of other (e.g., epigenetic) regulatory mechanisms is warranted.

Postnatal expansion of Kiss1 neurons—role of Kiss1 system in onset of puberty

Compelling evidence indicates that hypothalamic Kiss 1 system undergoes a complex pattern of neuroanatomical maturation and functional activation during the course of puberty. Functional relevance of such an activation of Kp signaling on timing of puberty was initially disclosed by the observations of a lack of pubertal maturation in human and mice harboring inactivating mutations of GPR54 or Kiss1 genes [8, 9]. Similarly a novel loss of functional mutation (p.F2725) in the GPR54 gene in a highly consanguineous family of Israeli–Muslim–Arab origin was associated with IHH [54]. Also previously only inactivating mutations in genes encoding human Kp receptor, (KISS1R), TAC3, TACR3 were known to cause IHH but recently Topaloglu reported IHH due to inactivating mutation of Kp in a large consanguineous family indicating functional Kp to be essential for reproduction in humans [55]. More recently, a study involving a central infusion of a specific antagonist during the pubertal transition has unequivocally documented that selective blockade of Kp actions disrupts the normal progression of puberty in the rat [56]. Conversely

repeated administration of Kp to immature rats accelerated the onset of puberty [57]. In vivo and ex vivo Kp are able to activate GnRH neurons, as demonstrated by c-fos detection and electrophysiological recordings and elicit GnRH secretion in sheep (in vivo) and by incubated rat hypothalamic explants (in vitro). A combination of expression and functional studies conducted mostly in rodents have clarified how Kp system participates in control of puberty in mammals; a multifaceted phenomenon that involves different maturational components. Initial data demonstrates that hypothalamic expression of Kiss1 increases during pubertal transition in the rat and monkey [19, 58], thus suggesting an elevation of its hypothalamic tone at the time of puberty. This was later confirmed by IHC studies that found a significant elevation in the number of Kp positive neurons at the AVPV during postnatal maturation [16]. Such combination of developmental changes revealed i) elevated Kptone ii) enhanced Kp signaling efficiency, iii) and GnRH/Gonadotropin (Gn) responses and iv) higher number of Kiss1 neurons and synaptic contacts with GnRH neurons.

More recent studies have focused on the mechanisms such as cascade of activational events and their regulation upstream of the Kiss1 neurons. In this context recent experimental evidence in mice suggests that the pubertal increase in Kiss1/Kp expression in the hypothalamus relies on some degree of oestrogenic input from the ovary—thus a positive feedback loop between the hypothalamus and ovary should be operative before puberty, implying Kiss1 neurons are not the triggers but rather oestrogen dependent amplifiers of GnRH neuron activity during the peripubertal period [18]. By the use of genetic engineering mice lacking oestrogen receptor α (ER- α) selectively in Kiss1 expressing cells, Mayer et al. [59] demonstrated that elimination of ER α mediated oestrogen actions in Kiss1 neurons resulted in marked activation of Kiss1 expression in the Arc during the peripubertal period, a phenomenon that was associated with a phenotype of signs of precocious puberty e.g. advanced vaginal opening. However such earlier activation of the gonadotropic axis in the model was followed by the arrest of later pubertal development and failure of first ovulation, which are likely due to defective expansion of the Kiss1 neuronal population at the AVPV.

Recently Guerriero et al. [60] used Kp54 agonist/antagonist in rhesus monkey to suggest that in addition to pubertal increases in Kp which contribute to pubertal increase in GnRH release there is a switch from ovarian steroid independent to dependent mechanisms in the response of GnRH to Kp.

Roles of Kiss1 neurons as excitatory afferents to GnRH neurons

A large number of studies have documented the ability of Kp to potently stimulate luteinizing hormone (LH) and to

some extent follicle stimulating hormone (FSH) secretion in virtually all species tested till date in adulthood [61, 62] including humans, where the stimulatory effects of Kp have been demonstrated even at very low doses. There is general consensus that the primary site of action of Kp is the GnRH neuron, as demonstrated by expression and functional study [56]. Thus, GnRH neurons have been shown to express GPR54, whereas the stimulatory effect of Kp in terms of Gn release is abrogated by pretreatment with GnRH antagonists. These observations, together with their direct projections to Gn RH neurons, solidly demonstrate that Kiss1 neurons are a key excitatory input to GnRH neuron system [63–65].

While the existence of direct projections of Kiss1 neurons to GnRH neurons is well documented, functional data strongly suggests that Kp may also partially operate indirectly on GnRH neurons to modulate Gn secretion. This has been pointed out by electrophysiological studies suggesting the i) influence of Kp signaling on glutamatergic and GABAergic (gamma amino butyric acidergic) transmission onto GnRH neurons [66]. Likewise studies in sheep have suggested a delicate ii) interplay between Kp and the orthologs of the gonadotropin inhibitory hormone (GnIH) in the dynamic control of GnRH secretion [67]. iii) Also important is its interplay of Kp with another cotransmitter-neurokinin B (NKB), whose physiologic relevance has been recently stressed by two human genetic studies [54, 66]. iv) Identification of prolactin sensitive GABA and Kp neurons in rostral hypothalamus suggests Kp neurons project effects of hyperprolactinaemia to GnRH neurons [67] with physiological significance in irregular cycles. Although Kp mainly acts on hypothalamus to stimulate gonadotropic axis studies in different species [26] document Kp can act directly on pituitary to evoke Ca responses and LH secretion however its physiological significance is not known.

Roles of the hypothalamic KISS1 system in the feedback control of gonadotropins by sex steroids

Among its various modulators sex steroids play a pivotal role in the dynamic control of GnRH and Gn secretion. The nature of sex steroid actions is in part sexually dimorphic and dependent on the physiological state. This is nicely illustrated by the fact that oestrogen (Og) can selectively stimulate Gn release at specific stages of the ovarian cycle (positive feedback) but does not elicit Gn secretion in males. Indeed in the female Og can be detected in the hypothalamus to regulate the tonic and preovulatory secretory modes of GnRH/Gn's [68] i) during the follicular phase. Moderate amounts of Og secreted by the ovaries helps to keep the tonic secretion of GnRH by negative feedback mechanism ii) At the end of the follicular phase, however an increase in circulating Og levels changes the pattern of secretion of

GnRH neurons triggering the preovulatory surge of GnRH/Gn's. Both positive and negative feedback mechanisms have been demonstrated to be made by neuronal ER α signaling [69]. Yet the lack of existence of ER α in GnRH neurons strongly suggests an intermediary pathway responsible for conveying the effects of Og to GnRH neurons—a function in which Kiss1 neurons have been proposed to play a prominent role [61, 62].

Role of Kiss1 neurons in positive feedback and the control of preovulatory surge

The fact that Kp signaling is essential for the preovulatory surge of Gn's has been suggested by the anovulatory state of Gpr54 and Kiss1 null mice [70], but also a recent data showing that blockade of Kp actions by the use of a specific antagonist disrupts the preovulatory surge of gonadotrophins [56], findings that recapitulate the previous results in a model of acute central immunoneutralization of Kp [36]. In any event compelling evidence has led to the belief that Kiss1 neurons at AVPV are important mediators for the Og actions on the generation of the preovulatory GnRH/Gn. While the experimental evidence supporting this contention is ample the following observations are highlighted—namely i) Og upregulates the expression of Kiss1 selectively at the AVPV via ER α ii) virtually all Kiss1 neurons at AVPV express ER α iii) Kiss1 neurons in the AVPV are activated as evidenced by c-fos expression, at the preovulatory period and during sex steroid primed LH surge iv) blockade of endogenous Kp in the preoptic area (POA) abolishes the proestrus LH surge. These data together with the proven existence of projections of Kiss1 neurons from AVPV to GnRH neurons makes a solid case for a crucial role of the population in the positive feedback effects of Og on GnRH/Gn's secretion. The molecular mechanism whereby Og upregulates the expression of Kiss1 at the AVPV have been recently evaluated using a genetically modified mouse model, engineered to lack ER α binding to consensus estrogen response elements (ERE) in DNA, called classical ER pathways, but that retains other (nonclassical) aspects of signaling. Through this model Gottsch et al. [71] demonstrated that Og acts via classical ER α -ERE pathways to stimulate Kiss1 expression at the AVPV. This is in contrast with the mechanisms for Og regulation of Kiss1 mRNA at the Arc and illustrates important molecular differences for the actions of Og in the regulation of these two populations of Kiss1 neurons.

In contrast to mice and rats in sheep and primates Kp neurons are only localized to Arc and POA. Here Arc appears to be responsible for both negative and positive feedback [23]. In adult ewe neurons Kiss1 mRNA in the caudal Arc rises during follicular phase and in rostral Arc at estrus when the LH surge occurs [23]. This suggests that the

Arc is responsible for modulating the positive steroid feedback in the ewe, during the estrus cycle, Kp can synchronize LH surges and during anestrus, administration of Kp can cause ovulation to occur, suggesting that when Kp levels are high enough they can cause the LH surge and they are therefore probably involved in relaying positive feedback to GnRH neurons [31]. Yet recent data suggest that Kiss1 neurons in sheep POA are also activated at the time of preovulatory surge and may also play a role in this phenomenon [72].

Role of Kiss1 neurons in mediating negative feedback control of gonadotropins by sex steroids

The mediator of steroid feedback has remained elusive for many years as GnRH neurons only possess ER β that doesn't play a role in feedback. Therefore neurons upstream of the GnRH neuron, which possess ER α , progesterone receptor (PR), and androgen receptor (AR) have been sought as possible mediators of steroid effects on GnRH release. Kp neurons express ER α , PR, AR and therefore have the potential to relay feedback effects on GnRH neurons. Opposite to their roles in AVPV of rodents Kiss1 neurons in the Arc, or in the equivalent infundibular region have been suggested to be crucial for mediating negative feedback actions of sex steroids on GnRH/Gn secretion in different species [23]. The most salient data supporting this contention involves removing sex steroids, which have been shown to elevate Kiss1 Mrna levels in the Arc whereby replacing Og or testosterone (Tn) prevented such increases. The effects of sex steroids are mediated via ER α and AR as demonstrated in mouse studies. As indicated above this is in contrast with the mechanism for Og regulation of Kiss1 expression at the AVPV. Also Kiss1 neurons in the Arc have been recently shown to coexpress NKB and dynorphin (Dyn) in rodents, sheep, monkey and humans [29, 73, 74].

Cellular and molecular mechanisms for Kp activation of GnRH neurons

Kp signaling through GPR54/KISS1R couples to Gq/11 to activate phospholipase C and increase inositol triphosphate (IP3) and diacylglycerol (DAG) levels in the cell [6, 75, 76]. The subsequent increase in intracellular calcium (Ca⁺⁺) and DAG activates protein kinase C and initiates a kinase phosphorylation cascade resulting in phosphorylation of ERK1/2. DAG also stimulates GnRH depolarization by activation of a nonselective cation channel (TRPC) and inhibition of an inwardly rectifying potassium channel (Kir) [64, 77, 78]. Desensitisation of GPR54/KISS1R is mediated by G protein coupled serine/threonine kinase2 (GRK2) and β -arrestin in a cell culture model [79].

Cotransmitters of Kiss1 neurons: prominent roles of NKB

It is now well documented that Kiss1 neurons, specifically in the Arc/infundibular region, coexpress NKB and Dyn in multiple species [80]. This has led to the recognition of the complex neuropeptide phenotype of formerly catalogued Kiss1 neurons, which have been now renamed KNDy (Kisspeptin, NKB, Dyn) neurons [80]. More interest generated after the identification of inactivating mutations in some of these pathways (i.e. NKB) as a possible cause of IHH in humans [55, 66]. This has resulted in a number of functional and expressional studies addressing the physiological roles of NKB and Dyn signaling in the control of GnRH secretion.

This neuronal population has been proposed, as a central node for integral control of GnRH secretion, not only by virtue of its ability to activate GnRH neurons via direct Kp stimulation, but also because of the capacity of NKB and Dyn to reciprocally and finely regulate such Kp output [42, 73, 74]. In this context, the physiological roles and mechanisms of actions of NKB in the control of GnRH secretion have been extensively investigated recently.

NKB is a member of the tachykinin family of peptides characterized by a common C-terminal substance sequence (Phe-X-Gly-Leu-Met-NH₂), encoded by TAC3 in humans or Tac2 in rodents [81]. NKB binds preferentially, to NK3R (encoded by TACR3), although some marginal agonistic activity of NKB has been detected at other tachykinin receptors, NK1R and NK2R [82]. However, demonstration that inactivating mutations in TAC3 and TACR3 genes leads to IHH in humans [65, 77], a phenotype, similar to that of Gpr54-null patients, prompted study of detailed effects of the activation of NKB pathways on Gn secretion and of the underlying mechanisms. Intriguingly, mice engineered to lack functional NK3R apparently display preserved fertility [83]. In fact compelling evidence from different species (including rodents) has been presented recently showing that NKB signaling in the Arc is under the control of sex steroids and is likely involved in the regulation of the pulsatile secretion of GnRH via autocrine modulation of Kp release.

Administration of senktide, a selective agonist of NK3R, has been shown to elicit LH release in rat, sheep and monkey [22, 84]. See review [85].

KNDy neurons on the Arc form a dense network around the 3rd ventricle, where individual neurons are profusely interconnected to each other [15]. In addition, there is evidence for the projection of such neurons onto the median eminence, where KNDy fibers appear to be in the vicinity of GnRH nerve terminals [86]. Such neuroanatomical features together with the effects of NKB described here, have paved the way for the analysis of specific role of this system in the control of GnRH pulses. The experimental evidence available clearly suggests that NKB acts primarily on KNDy neurons to indirectly modulate the pulsatile release of GnRH

and thereby Gn secretion [42, 73, 74]. In support of this autoregulatory loop, KNDy neurons abundantly express NK3R and central infusion of senktide rapidly activates c-fos expression in Kiss1 expressing neurons [22]. In contrast GnRH neurons express little or none NK3R and are not rapidly activated upon central senktide administration [15]. Anyhow the fact that NKB acts within hypothalamus to elicit LH secretion is documented by the observation that this stimulatory action is blunted by pretreatment with a GnRH antagonist [22].

Roles of Kiss1 neurons in the metabolic control of reproductive axis

Reproduction is a metabolically demanding function that requires sufficient levels of energy stores to proceed [87]. This is more evident in mammalian females because of pregnancy and lactation, although reproductive function in male mammals is also sensitive to conditions of metabolic stress [87]. Accordingly, a sophisticated network of regulatory signals linking metabolism and reproductive function has evolved to ensure such a functional connection. Indeed GnRH secretion is influenced by a number of metabolic signals e.g., glucose, insulin, leptin and ghrelin [87, 88], which nonetheless may not directly act on GnRH neurons. A paradigmatic e.g. is leptin, a signal of energy balance from adipose tissue that is indispensable to normal puberty onset and fertility but whose functional receptors are not expressed on GnRH neurons [89]. Therefore it is tenable that upstream afferents to GnRH neurons are primarily responsible for the integration of, at least part of this metabolic information directed to GnRH neurons. Kiss1 neurons are likely to be an important element of the central circuits driving (directly or indirectly) nutritional/metabolic information to GnRH neurons.

Regulation of the Kiss1 system by the metabolic status

Conditions of negative energy balance as defined by the reduction of a metabolizable substrate or in the capacity to use them, have a considerable impact on the reproductive function. In humans, situations of energy shortage, e.g. anorexia or diabetes, negatively affect fertility [90, 91]. In this context, several studies have documented a clear impact of conditions of undernutrition or metabolic stress on Kiss1/Kp expression in the hypothalamus. Thus, mice subjected to fasting display a significant reduction in hypothalamic Kiss1 mRNA levels, which is already evident after 12 h and precedes the decline in GnRH expression [92]. Similar results have been obtained in pubertal rats as well as monkeys, where a significant decline in Kiss1 mRNA levels has been reported after short term fasting [93–95]. Similarly chronic subnutrition during puberty has been shown to

reduce Kiss1 Mrna level in Arc in female rats [95–98]. In good agreement during lactation when the metabolic cost of milk production causes a state of negative energy balance in the dam that leads to decreased GnRH release and anovulation. Kp/NKB content in the Arc, as well as Kiss1 Mrna levels in the Arc and AVPV, are significantly lower in the rat [86, 99]. Likewise situations of negative energy balance due to uncontrolled diabetes in rats evoke suppression of Hypothalamic expression of Kiss1, which was associated to a state of HH [100, 101].

The functional relevance of the above changes in conditions of negative energy balance is reinforced by the observation that repeated central injections of Kp10 to female rats with puberty arrest due to chronic subnutrition was able to restore vaginal opening (an external sign of puberty) and to induce potent gonadotropic and oestrogenic responses despite the persistent caloric restriction [94]. In the same line Kp10 administration to uncontrolled diabetic male rats rescued the hypogonadotropic phenotype of these animals [96, 97]. Taken together these findings suggest a role of Kiss1 system as a putative sensor of the energy state of the organism and relay node of transmission of metabolic information onto GnRHneurons.

Kiss1 neurons as putative mediators of the positive effects of leptin on reproduction

Given the permissive role of leptin in the metabolic control of puberty onset and reproductive function, this system has been evaluated in different species. In this context recent efforts have been devoted to elucidate the molecular mechanisms whereby leptin modulates Kiss1 expression and reproductive function namely Crebcoactivator (TORC1) and mTOR [95–98]. Considering the repeated observations of leptin induced changes in Kiss1 Mrna levels in the hypothalamus in different species, it remains possible that leptin effects on Kiss1 neurons are indirect, that is they are achieved by engaging some of their potential neuronal afferents. Indeed in the same study [99], it was demonstrated that the ventral premammillary nucleus (PMV), an hypothalamic area with abundant expression of LepR sends projection to Kiss1 and GnRH neurons and appears to play an indispensable role in mediating leptin effects on the gonadotropic axis as evidenced by its stimulatory effects on that of unilateral restoration of LepR phenotypes of PMV in LepR null mice [100, 101]. The phenotypes of PMV neuronal elements mediating such a function and whether these interact with Kiss1 neurons are yet to be elucidated. Of the latter it is noted that despite the rescued mice, no obvious differences in terms of relative levels of Kiss1 expression were detected in the Arc and AVPV. While this may argue against an interaction between PMV and Kiss1 neurons, such a lack of change in Kiss1 expression is somewhat at odds with the

predictable modifications induced by the changing levels of sex steroids upon Kiss1 expression.

Melanin concentrating hormone (MCH) may also provide a link between energy status and reproduction and this hormone is upregulated during fasting and can directly inhibit the action of Kp on some GnRH neurons [102].

Human studies and therapeutic implications

With the greater understanding of the biology of Kp signaling it becomes increasingly tempting to speculate therapeutic uses in the treatment of infertility especially in HH.

Studies in normal physiology

1) Kp on Gn Secretion

When administered to healthy male subjects iv infusions of Kp54 results in a significant increase in plasma LH, FSH and Tn [103].

- i) Sc injection of Kp54 to healthy premenopausal female subjects elicits a marked rise in LH which is most pronounced in the preovulatory phase of the menstrual cycle [104].
- ii) The first study using Kp10 in humans was by George et al. [105] who used Kp10 in healthy men and found iv Kp10 both as boluses and infusion with as low a dose as 1 µg/kg for 22.5 h markedly increased LH pulse frequency and pulse size, leading to an increase in LH, Tn and that no tachyphylaxis was observed in this short duration.
- iii) Advantages of using Kp10 over Kp54 being that Kp54 is cleaved from 145aa precursor polypeptide and is further processed to 14, 13 and 10aa sequences. Although Kp10 has intrinsic bioactivity similar to the longer Kp fragments [106], it is also characterized by a shorter half life and more rapid onset of action after iv administration in rodents [107]. Kp10 also has greater potential for pharmaceutical development because both agonists [108] and antagonists [108, 109] have been developed based on its decapeptide sequence. For details see review [85]
- iv) Subsequently Jayasena et al. [110] using Kp10 in both healthy males and females have shown a sexually dimorphic effect in humans—stimulating gonadotropin release in healthy men with iv bolus with low dose as of 0.3 and 1 nmol/kg but being ineffective with the maximum doses s.c. or iv or by iv infusion (10 nmol/kg, 32 nmol/kg, 720pmol/kg/min) in follicular phase however increasing LH and FSH during the preovulatory phase with iv bolus 10 nmol/kg.

2) Kp in placental invasion

Kp is found i) in high concentration in 3rd trimester of pregnancy with peripheral levels being 7,000 fold greater than nonpregnant state [111] and is also found in amniotic fluid [112] but exact significance is unknown, however Kp10 inhibits trophoblast migration in human placental explants [113]. Plasma levels of Kp ir is increased in gestational trophoblast disease GTD [114, 115]. It is speculated that Kp may act to regulate trophoblast invasion of uterine tissue. However as patients with inactivating mutations of kp receptor are able to undergo normal pregnancy after treatment with GnRH/Gn's [116]. Role of Kp in pregnancy remains controversial.

Prospective therapeutic roles

- i) Initially Jayasena et al. tried twice daily s.c injection of Kp in functional hypothalamic amenorrhoea (HA) due

to low body weight and found Kp effectively stimulated Gn's. This effect was marked following first injection but significantly diminished after 2 weeks of treatment [117]. However biweekly administration of Kp resulted in a sustained Gn response to Kp treatment [118], and that in women with HA, the LH response was four fold greater than that of healthy females.

- ii) Also recently Young et al. [119] reported restoration of pulsatile LH secretion in patients with NKB signaling deficiencies by continuous Kp infusion e.g. loss of function mutations in NKB (TAC3) or its receptor (TAC3R), indicating that Kp on its own is sufficient to stimulate pulsatile GnRH secretion.

- iii) Also Kp resets the hypothalamic GnRH pulse generator in men—the first known agent capable of resetting the GnRH clock in man. Chan et al. [120] found that single bolus injection of Kp 10 induced immediate LH pulse regardless of timing of previous endogenous pulses. Comparison of morphology of Kp induced LH pulses

Fig. 1 Normal physiology and the pathophysiological process underpinning of Kissi neuron involvement in obesity hypothesis (Courtesy Ref [121] George et al., with permission)

in healthy men with that of GnRH induced LH pulses in men with isolated GnRH deficiency suggests that a single iv bolus of Kp triggered sustained GnRH release lasting approx 17 h and it reset the GnRH pulse generator as it not only induced immediate LH pulses but also delayed next endogenous pulses by an interval equaling approximately the normal interpulse interval.

- iva) Role in Male Hypogonadism in Obesity and Type2 Diabetes—George et al. [121] proposed that just as in animal studies Kp may be the missing link between insulin, leptin, Og, and inflammation with hypogonadism. GnRH neurons which lack corresponding receptors egObmRNA/Er α , it seems Kp constitutes the missing link about conveying information about whole body energy balance to GnRH neuronal system. From a translational perspective there is a potential for pharmacological therapies to be developed to address hypogonadism—Fig. 1. A number of Kp analogues are being developed with agonistic and antagonistic activities. Kp agonists constitute putative therapeutic targets for low circulating Tn seen in obesity and diabetes mellitus. Current treatment of hypogonadism with testosterone tends to decrease sperm production. Pharmacological modulations with Kp agonists will potentially be free of this adverse effects.
- ivb) Similarly only a subgroup of obesity subjects suffer from HH. Animal models offer similar explanations of reduced leptin signaling leading to reduced GnRH secondary to a decreased Kp expression [122].
- v) By extending the effect of metabolic control of leptin via Mtor in regulating Kp expression the uses may be extended to those of Mtor e.g. recently Mtor pathways proposed to play a major role as link between obesity and carcinogenesis [123] and that Mtor inhibitors are being widely used in treatment of cancer and organ transplant patients [124] and chronic treatment with these drugs have been reported to impair gonadal functions—Novel insights may be gained for the impact on reproductive function by deeper understanding and impact of Mtor antagonists of clinical use in some types of cancer and organ transplantation [125–127] and also to understand pathophysiology of anorexia nervosa and whether Kp may be curative or not.
- vi) Also recently rare missense mutations and single nucleotide polymorphisms (SNP's) of the Kp system were associated with puberty onset and two gain of functional mutations of KISS1 and KISS1R are implicated in the pathogenesis of GnRH dependent precocious puberty [128]—hence Kp antagonists may have role in precocious puberty treatment.
- vii) Thus considering above Kp10 may have a role in infertility, HA, HH, Delayed puberty, and prostatic or metastatic cancers [129] and even in resistant PCOS
- viii) Another possible role Kps may have is in pathophysiology of endometriosis [130].

Conclusions

Thus Kp/KISS1 system is involved in control of brain sexual maturation, puberty initiation, control of GnRH secretion by sex steroids modulating positive and negative feedback of E2. Kp agonists specially Kp10 may have multiple therapeutic benefits like in infertility, HA, HH, metastatic and prostate cancers, Some unanswered questions remain, like which molecules act upstream of kiss1 to regulate expression and what are the mechanisms by which two populations of Kp neurons (AVPV and ARC) are differentially regulated by sex steroids.

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