Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility

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Abstract | The neurohormonal control of reproduction involves a hierarchical network of central and peripheral signals in the hypothalamic–pituitary–gonadal (HPG) axis. Development and function of this neuroendocrine system is the result of a lifelong delicate balance between endogenous regulators and environmental cues, including nutritional and metabolic factors. Kisspeptins are the peptide products of *KISS1*, which operate via the G-protein-coupled receptor GPR54 (also known as Kiss1R). These peptides have emerged as essential upstream regulators of neurons secreting gonadotropin-releasing hormone (GnRH), the major hypothalamic node for the stimulatory control of the HPG axis. They are potent elicitors of gonadotropin secretion in various species and physiological settings. Moreover, Kiss1 neurons in the hypothalamus participate in crucial features of reproductive maturation and function, such as brain-level sex differentiation, puberty onset and the neuroendocrine regulation of gonadotropin secretion and ovulation. Cotransmitters of Kiss1 neurons, such as neurokinin B, with roles in controlling the HPG axis have been identified by genetic, neuroanatomical and physiological studies. In addition, a putative role has been proposed for Kiss1 neurons in transmitting metabolic information to GnRH neurons, although the precise mechanisms are as yet unclear. In this Review, we present the major reproductive features of kisspeptins, especially their interplay with neurokinin B and potential roles in the metabolic control of puberty and fertility, and suggest new avenues for research.

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Introduction

Reproduction is indispensable to perpetuate species. As such, this function is under the control of complex regulatory networks that are sensitive to numerous endogenous signals and environmental modifiers. Hormonal control of reproduction primarily relies on the dynamic interplay between signals originating from three major sources: the anterior hypothalamus, where a small group of neurons synthesize the decapeptide gonadotropin-releasing hormone (GnRH); the adenohypophysis, where gonadotrope cells are stimulated by GnRH to synthesize and release pituitary gonadotropins, luteinizing hormone and follicle-stimulating hormone; and the gonads, which, in addition to generation of gametes from puberty onwards, respond to the trophic actions of gonadotropins with increased secretion of sex-steroid and peptide hormones.[1–3] In turn, gonadal hormones feed back to the hypothalamus and pituitary to regulate secretion of GnRH and gonadotropin.[1–3] These elements comprise the hypothalamic–pituitary–gonadal (HPG) axis (also known as the gonadotropic axis).

GnRH neurons play a crucial hierarchical part in this axis to enable input of a large array of central and peripheral signals, mainly indirectly, into the GnRH system. In turn, GnRH operates as the final output pathway for regulatory actions upon the downstream elements of the reproductive axis.[1,3,4] Notably, the anatomical organization and the function of key elements of the HPG axis undergo substantial changes during prenatal and postnatal maturation. Developmental modifications, therefore, constitute a continuum that at brain level includes the process of brain sex-differentiation, puberty onset and, dependent on the species, reproductive senescence.

Reproductive maturation and function are tightly coupled with other essential body functions, including regulation of body weight and energy use and storage.[5–7] Such a close relationship is founded on complex interactions of multiple neuroendocrine integrators that affect metabolic and reproductive pathways. The functional relevance of this phenomenon manifests in conditions of energy imbalance and metabolic stress, in which perturbations of puberty onset and fertility are frequently observed, and in some forms of gonadal or reproductive disease that are associated with severe metabolic impairment, such as the polycystic ovarian syndrome.[8] Although the nature and mechanisms of action of the signals responsible for the metabolic–reproductive interplay have been partly explained, our understanding remains incomplete.

Our knowledge of the physiological systems responsible for the control of the HPG axis in various species, including humans, is growing. Much has been learnt

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about the neuronal circuits and signals involved in the dynamic regulation of the hypothalamic secretion of GnRH and its modulation by different hormonal and environmental modulators. An important finding was the recognition of the reproductive role of a family of peptides, globally termed kisspeptins, that were initially regarded as metastasis suppressors.[9,10] In this Review, we present the milestones in the field of kisspeptin physiology, paying especial attention to developments in two particular related areas: the putative interplay of kisspeptins and their cotransmitters, especially neurokinin B, in the central control of GnRH neurons; and the possible role of Kiss1 neurons as conduits for the metabolic regulation of puberty and fertility, with emphasis on intermediate regulatory signals and mechanisms involved. We use *KISS1* and *Kiss1* to indicate the primate and nonprimate genes, respectively, and their peptide products are referred to as kisspeptins (abbreviated to Kp with a numeric extension if the number of amino acid residues needs to be indicated).[11] In addition, although KISS1R is the approved symbol for the primate receptor,[11] we have adopted the more widely used GPR54 (encoded by *GPR54*) or Gpr54 (encoded by *Gpr54*) for primates and nonprimates, respectively. Finally, as most of the experimental data we discuss have been obtained in nonprimate species, we use the term Kiss1 for global reference to the ligand–receptor system.

Key points

- Kisspeptins, the ligands of the G-protein-coupled receptor GPR54, are encoded in humans by the *KISS1* gene and operate as potent stimulators of gonadotropin-releasing hormone (GnRH) in the reproductive brain
- Kisspeptin pathways are involved in the control of crucial aspects of reproductive maturation and function, including puberty onset, sex-steroid control of gonadotropin secretion and ovulation
- Discrete populations of Kiss1 neurons exist in the arcuate nucleus of the hypothalamus in humans and nonprimates and in the rostral periventricular area in rodents; arcuate Kiss1 neurons coexpress neurokinin B
- Hypothalamic expression of Kiss1 is sensitive to negative energy balance (potentially including obesity) and probably acts as a sensor and transmitter of metabolic information to GnRH neurons
- The signals and mechanisms underlying metabolic control of Kiss1 neurons are poorly defined; a role for leptin is proposed, but its mechanisms of action are not yet clear
- Interplay between the Kiss1 system and various molecular mediators and other neuropeptide pathways seems important for energy homeostasis, but data are inconsistent and need to be interpreted with caution

Kisspeptin signaling in the brain

Kisspeptins were originally identified in cancer biology as the products of the metastasis suppressor gene *KISS1*, which led to the initial name of metastin.[12] The canonical receptor for kisspeptins, GPR54, was first discovered in 1999 as an orphan receptor;[13] in 2001, three independent groups identified it as a receptor for *KISS1*-derived peptides.[12,14,15] Kisspeptins are thought to exist in different lengths—Kp-54 (metastin), Kp-14, Kp-13 and Kp-10—that are generated by cleavage of a common 145 amino acid precursor. All kisspeptins share a 10 amino acid carboxyterminal sequence, which is enough to fully activate GPR54, and in humans contains the distinctive Arg–Phe–NH_2 motif, characteristic of the large RF-amide peptide superfamily.[15]

In 2003, two independent studies documented the connection between the Kiss1 system and reproductive function, on the basis of findings from clinical studies.[16,17] Patients bearing loss-of-function mutations in *GPR54* had isolated hypogonadotropic hypogonadism, with absence of puberty and infertility. This phenotype has been reproduced in mice lacking functional *Gpr54* or *Kiss1* genes.[18] Recognition of the reproductive dimension of Kiss1 signaling by means of human genetics and functional genomic studies boosted the interest of reproductive physiologists. They aimed to decipher the biological roles and modes of action of kisspeptins in the control of different features of the development and function of the HPG axis, mainly in animal models and to some extent in cellular models. As a result, during the past 7 years, kisspeptins have been thoroughly analyzed and are now regarded as the most potent naturally occurring stimulators of GnRH and, therefore, gonadotropin secretion.[9,10]

A wealth of experimental data show that the primary site for activation of the HPG axis by kisspeptins is the hypothalamus, where these peptides can directly activate GnRH neurons.[9,10] The evidence can be summarized as follows: GnRH neurons express GPR54;[19] kisspeptins induce *Fos* expression (which is a sign of early activation) in GnRH neurons;[19] GnRH antagonists ablate release of gonadotropins evoked by kisspeptins;[19,20] kisspeptins exert potent depolarizing effects on GnRH neurons, as measured by voltage recordings in hypothalamic slice preparations;[21–23] kisspeptins stimulate GnRH release from hypothalamic explants *ex vivo* and into the cerebrospinal fluid *in vivo*;[24,25] and blockade of kisspeptin signaling by the use of a specific antagonist decreases GnRH neuronal firing and the pulsatile release of GnRH.[26] These direct actions do not, however, preclude additional indirect stimulation via modulation of excitatory afferents to GnRH neurons,[23] nor the possibility of subordinated actions directly at the pituitary level to stimulate secretion of gonadotropes.[27]

Further understanding of kisspeptin physiology has come from anatomical studies, which have shown the presence of the elements of the Kiss1 system in key hypothalamic circuits involved in the central control of reproduction. In addition, studies in several species have shown the putative expression of *Kiss1* and *GPR54* in a variety of tissues and brain areas, including the ovaries, testes, placenta, pancreas and pituitary.[9,28] Given the scope of this Review, however, we are able to only briefly summarize the hypothalamic and central distributions of this ligand–receptor system; we pay special attention to data from rodents and present some findings from sheep and primate models as well.

Several neuroanatomical studies in mice and rats have revealed two major areas of *Kiss1* mRNA expression within the hypothalamus: the arcuate nucleus and the anteroventral periventricular nucleus/rostral

periventricular nuclei (AVPV/PeN).[29,30] In the latter, *Kiss1* expression is markedly sexually dimorphic and much more abundant in female than male adults.[31] *In situ* hybridization has also shown *Kiss1* mRNA at lower levels in the preoptic nucleus, the amygdala and the bed nucleus of the stria terminalis.[32] Cravo *et al.*,[33] who used a *Kiss1*-Cre/β-galactosidase reporter mouse line, provided evidence for a broad expression pattern for *Kiss1*, including the lateral septum, anterodorsal preoptic nucleus, medial preoptic nucleus, ventromedial nucleus of the hypothalamus, medial nucleus of the amygdala and nucleus of the solitary tract. The specificity of *Kiss1*-driven expression of Cre recombinase in this transgenic line is, however, yet to be fully proven. In sheep and primates, including humans, prominent expression of *Kiss1* or *KISS1* mRNA has been unambiguously demonstrated in the arcuate nucleus.[34–36] The presence of a more-rostral population of neurons expressing *Kiss1* in these species is being investigated (see below). *In situ* hybridization studies have shown the presence of Kiss1 neurons in the preoptic area of sheep,[36] whereas, to the best of our knowledge, presence of *KISS1* mRNA has not been reported in analogous regions in the hypothalamus of primates, including humans.

Immunohistochemistry analyses in rodents have shown kisspeptin immunoreactivity in several brain areas, including the hypothalamic arcuate nucleus and AVPV/PeN and extrahypothalamic regions.[37] A limitation of early immunohistochemistry studies was cross-reactivity of several kisspeptin antibodies with other RF-amide peptides. Later assessments in mouse hypothalamus, however, largely overcame this issue and have shown extensive overlapping of mRNA and protein data.[37,38] Some slight discrepancies between *in situ* hybridization and immunohistochemistry results have, however, been noted. For instance, kisspeptin immunoreactivity but not *Kiss1* mRNA has been detected in the dorsomedial nucleus of rodents.[37] In monkeys, prominent kisspeptin immunoreactivity was initially identified only in the arcuate nucleus,[39] although later studies have suggested some degree of peptide expression in the preoptic area. Neuroanatomical studies in humans indicate that, in addition to the abundant population of Kiss1 neurons in the arcuate nucleus, discernible populations of kisspeptin-positive cells are present in the periventricular area, embedded in a dense plexus of fibers with strong kisspeptin immunoreactivity.[40]

Location analyses have also addressed the distribution of kisspeptin fibers and the expression of GPR54 in different brain areas. In a rat study done to investigate projection patterns of arcuate nucleus Kiss1 neurons, in which neurokinin B was used as a surrogate marker, bilateral arcuate nucleus, median eminence and periventricular projections were seen.[41] In addition, other hypothalamic areas, such as the retrochiasmatic area and the bed nucleus of the stria terminalis, were shown to be targets.[41] Some of these projection patterns, however, need to be fully characterized and confirmed in other species. In an elegant neuroanatomical study that used anterograde and retrograde tracing techniques, the projections of the two major populations of Kiss1 neurons were mapped in the brains of female mice. Thus, these analyses have documented the projection of Kiss1 neuron populations to various hypothalamic and extrahypothalamic (for example, limbic) nuclei, and shown that a subset of Kiss1 neurons in the rostral arcuate nucleus project to the preoptic area, where GnRH neuronal cell bodies are located, as previously reported for Kiss1 neurons in AVPV/PeN.[42]

By contrast, mapping of GPR54 distribution has lagged behind, due in part to the lack of reliable antibodies. Nevertheless, various studies that used a *Gpr54*-driven lacZ knock-in mouse line have revealed expression of *Gpr54* in the following brain areas: the dentate gyrus of the hippocampus, septum, rostral preoptic area, anteroventral nucleus of the thalamus, posterior hypothalamus, periaqueductal grey, supramammillary and pontine nuclei, and dorsal cochlear nucleus.[43] Previously, *in situ* hybridization studies had shown that GnRH neurons in rat hypothalamus express *Gpr54*.[19]

Overall, the neuroanatomical data have improved understanding of the physiological modes of action of kisspeptins in the control of GnRH secretion. In this context, direct appositions between kisspeptin fibers and GnRH cell bodies have been demonstrated in mice, which supports a direct action of kisspeptins on GnRH neurons.[38] In rodents, kisspeptin projections to GnRH neurons seem to originate mainly from the AVPV/PeN, although projections in the arcuate nucleus have been noted, albeit rarely.[42] In addition, immunohistochemistry studies in monkeys have shown that kisspeptin fibers are in close proximity to GnRH terminals in the median eminence, which suggests a role for kisspeptin afferents, probably stemming from the arcuate nucleus, in the control of GnRH release at this site.[39] This theory is supported by the ability of Kp-10 to stimulate GnRH secretion by mediobasal hypothalamic preparations devoid of GnRH cell bodies in mice.[44] The number of synaptic contacts between Kiss1 and GnRH neurons in the median eminence seems, however, to be surprisingly low, which suggests interneuronal or even nonsynaptic communication between these two neuronal populations. On the other hand, the presence of abundant bilateral kisspeptin projections within the arcuate nucleus, which seem to form a dense network of kisspeptin fibers surrounding and making contact with Kiss1 cell bodies,[45] supports the existence of autosynaptic feedback regulatory mechanisms, whose relevance has been highlighted by data from studies of neurokinin B.

Kiss1 and the control of reproduction

The recognition of the pivotal roles of Kiss1 neurons at discrete hypothalamic nuclei in the central drive of GnRH secretory activity and, therefore, of the HPG axis has led to the systematic analysis of their involvement in key aspects of the maturation and function of the reproductive system. The results of these analyses have substantially expanded understanding of fundamental reproductive phenomena. Although, for the sake of

conciseness, not all facets of kisspeptin physiology will be thoroughly discussed here, we present an overview of the major features of kisspeptins as gatekeepers of reproductive function to put in context the more-in-depth discussions of specific facets of kisspeptin functions presented in later sections of this Review.

Brain sex-differentiation

Key features of the maturation and function of the reproductive axis, including the timing of puberty and the patterns of tonic versus cyclic gonadotropin secretion, are sexually dimorphic.[9] Complete manifestation of these dimorphisms relies on a complex program of early differentiation events that shapes the functional organization of the hypothalamic circuits governing GnRH secretion. This phenomenon is driven to a great extent by the actions of sex steroids on the developing brain.[46] Evidence strongly supports Kiss1 neurons being targets of the organizing effects of sex steroids during early (critical) periods. Thus, Kiss1 neurons are also surrogate markers for and eventual components of the mechanisms of sexual differentiation of the brain.[47] These roles are reflected by populations of Kiss1 neurons at the AVPV/PeN being more abundant in females than males, a phenomenon that has been linked to the generation of the preovulatory surge of gonadotropins.[31]

The above dimorphism in rodents is shaped by early exposures to sex steroids, which is demonstrated by reversal of the sex-specific developmental program of this neuronal population after perinatal androgenization of females or gonadectomy of males.[47] Notably, durable changes in the Kiss1 neuronal population in the AVPV/PeN induced by early manipulations of the sex-steroid milieu are coupled with functional alterations, in terms of gonadotropin secretion in adulthood. Hence, androgenized female rats, which have a male-like population of AVPV/PeN Kiss1 neurons, have no preovulatory surges of luteinizing hormone, even if they are properly primed with sex steroids. This evidence, however, is circumstantial and does not show conclusively that Kiss1 neurons are primarily responsible for the functional sex dimorphism of the gonadotropic axis. Similarly, the mechanisms by which the structural sex differences of Kiss1 neuronal populations arise remain ill-defined.

Onset of puberty

The role of kisspeptin signaling in the control of onset of puberty was initially suggested by the absence of puberty in humans and mice with inactivating mutations of *GPR*54 or *Gpr54*, respectively.[17] Models of congenital absence of GPR54 did not, however, discriminate between early organizing defects, due to the absence of kisspeptin signaling or the lowering of sex-steroid concentrations, and the actual lack of Kiss1 excitatory inputs during the pubertal transition. Many experimental data indicate an essential role for kisspeptins in the timing of puberty and that the Kiss1 system undergoes a complex pattern of activation during pubertal maturation. This event probably involves increased hypothalamic expression of *Kiss1* and GnRH responsiveness to kisspeptins, as well as important plastic changes in the populations of Kiss1 neurons, especially those in the AVPV/PeN in rodents, whose number and synaptic contacts with GnRH neurons seem to increase substantially during puberty.[48,49] Studies in mice suggest that the expansion of Kiss1 neuronal population at the AVPV/PeN during puberty is driven by the precedent increase in ovarian-derived estradiol concentrations. This hypothesis implies that rather than triggering puberty, Kiss1 neurons operate as estrogen-dependent enhancers of the secretory activity of GnRH neurons during pubertal maturation.[49] Hence, kisspeptin-independent mechanisms, responsible for the initial rise of estrogen, would play an important part in early stages of puberty. The physiological relevance of this hypothesis in rodents and other mammals is yet to be fully validated, but kisspeptins clearly have an essential role in signaling at the time of puberty, which is supported by the fact that selective antagonization of kisspeptin actions leads to delayed puberty onset in female rats.[50]

Sex-steroid feedback

Sex steroids have an essential role in the feedback regulation of GnRH and gonadotropin secretion. The feedback actions of these steroids are predominantly inhibitory (negative feedback), although in adult females, estrogens can evoke the release of GnRH and gonadotropins at specific stages of the feedback cycle (positive feedback). Such differential regulation translates into different modes of secretion of GnRH and gonadotropin: tonic (in males and females) versus surge-like secretion (selectively in females).[51] Estrogens that originate from the ovary or that are locally produced after aromatization of testosterone, play essential parts in positive and negative feedback loops, mainly via signaling by estrogen receptor α.[52] GnRH neurons seem, however, to lack functional estrogen receptor α, which suggests that interneuronal pathways transmit sex-steroid feedback actions to GnRH neurons. Evidence indicates that Kiss1 neuronal populations participate in these phenomena through nucleus-specific specialization: arcuate nucleus Kiss1 neurons participate in negative feedback events, whereas those in the AVPV/PeN, at least in rodents, seem to have a dominant role in conveying positive feedback effects of estradiol and, therefore, are essential for the generation of the preovulatory surge of gonadotropins.[9,10]

The main evidence linking Kiss1 neurons in the arcuate nucleus and negative feedback can be summarized as follows: elimination of sex steroids by gonadectomy increases *Kiss1* mRNA levels in the arcuate nucleus; sex-steroid replacement prevents the rise of *Kiss1* expression in this nucleus after gonadectomy; and the absence of estrogen receptor α or androgen receptor prevent the rise of *Kiss1* mRNA levels at this site after gonadectomy.[29,30] In accordance, Kiss1 neurons in the arcuate nucleus express estrogen receptor α and androgen receptor, and a mouse model of estrogen-receptor-α ablation in Kiss1-expressing cells showed the relevance of signaling via this receptor in arcuate nucleus Kiss1 neurons

for mediation of the negative feedback on GnRH and gonadotropin secretion.[53] The functional relevance of this pathway is further, albeit indirectly, emphasized by the observation that *Gpr54* knockout mice do not display the usual rise in circulating gonadotropin concentrations after gonadectomy.[54] Similarly, increases in luteinizing hormone secretion after gonadectomy are blunted in rats and mice pretreated with a selective kisspeptin antagonist.[26] On the basis of expression analyses in models of gonadectomy or menopause in other animal species (for example, sheep, monkeys and humans), it is proposed that mediation of negative feedback actions of sex steroids via Kiss1 neurons in the arcuate nucleus is highly conserved across mammals.[9,10]

Preovulatory gonadotropin surge

In contrast to their role in the arcuate nucleus, Kiss1 neurons in AVPV/PeN have been linked to the positive feedback effects of estrogen in female rodents. The experimental evidence supporting this association can be summarized as follows: estrogen stimulates the expression of *Kiss1* selectively in the AVPV/PeN; Kiss1 neurons in these nuclei are activated during the preovulatory period and following sex-steroid induction of luteinizing hormone surges; and immunoneutralization of endogenous kisspeptins in the preoptic area abrogates the preovulatory luteinizing hormone surge.[55] In addition, given that almost all AVPV/PeN Kiss1 neurons express estrogen receptor α, which is vital for surge generation, and their projections to GnRH neurons, this Kiss1 neuron population might play an essential part in the generation of the preovulatory surge of gonadotropins. This theory is in keeping with initial observations of the anovulatory state of *Gpr54* and *Kiss1* knockout mice[56] and is supported by the prevention of the preovulatory surge after antagonization of GPR54.[50] Further evidence comes from the disruption to ovulation in mice after selective ablation of estrogen receptor α from *Kiss1*-expressing cells. This effect is probably due to impairment of development, function or both of Kiss1 neurons in the AVPV/PeN.[53]

Besides their roles as transcriptional regulators of *Kiss1*, sex steroids are also important modulators of GnRH/gonadotropin responses to kisspeptins. For instance, activation of estrogen receptor α in female rats increases the responsiveness of luteinizing hormone to Kp-10, especially in the presence of activated progesterone receptors. This effect probably contributes to the generation of full preovulatory gonadotropin surges.[57,58]

Reproductive senescence

Changes in the hypothalamic Kiss1 system have been reported in relation to reproductive aging. The nature and magnitude of such changes, however, are dependent on the species and patterns of age-related reproductive collapse. Thus, in female rodents, where reproductive senescence is primarily driven by a central failure of the HPG axis, defined by defective GnRH and gonadotropin secretion, decline in hypothalamic expression of *Kiss1* is seen in old females. Administration of exogenous kisspeptins restores normal preovulatory surges in old rats.[59,60] Reduced kisspeptin tone in the hypothalamus is, therefore, at least partly causative in the decline of gonadotropic function in ageing rodents. By contrast, in humans and monkeys, where reproductive senescence manifests initially as ovarian failure, the loss of sex-steroid secretion associated with menopause evokes a rise in *KISS1* expression in the arcuate nucleus, which seems to be mechanistically relevant for the postmenopausal rise of gonadotropin levels.[34]

Roles of neurokinin B

Extensive analyses of the Kiss1 system have been done to elucidate the effects and modes of action of kisspeptins upon downstream elements of the HPG axis —mainly GnRH neurons— and to address the signals and mechanisms involved in regulation of kisspeptin release. Interest on the latter has increased substantially, and the emerging data are shaping a new model for the control of Kiss1 neuron activity.[61] Investigations on this front have centered on the elucidation of neurotransmitters coexpressed with kisspeptins at specific neuronal populations. Neurokinin B and dynorphin A have been revealed as partners and potential autoregulators of kisspeptin secretion. Expression analyses have conclusively demonstrated that colocalization of Kiss1, neurokinin B and dynorphin A is a specific feature of the Kiss1 neuron population in the arcuate nucleus, and the collective term KNDy neurons has been coined in recognition of this neuropeptide diversity.[61]

Goodman and co-workers[62] used immunohistochemistry to show neurokinin B and dynorphin A coexpression with Kiss1 neurons in the mediobasal hypothalamus of ewes. This phenomenon seems to be conserved in different species. Thus, colocalization of mRNA encoding dynorphin A and neurokinin B has been demonstrated in the arcuate nucleus of goats and mice (encoded by *Pdyn* and *Tac2*, respectively, in these species) by *in situ* hybridization;[63,64] coexpression of neurokinin B and kisspeptins has also been shown by immunohistochemistry in monkeys.[65]

Indirect evidence suggests that KNDy neurons exist in the rat arcuate nucleus.[66] Of note, coexpression of the neurokinin B receptor (encoded by *Tacr3*) has also been reported in KNDy neurons in mice.[63] Whether the dynorphin receptor, κ-opioid receptor (encoded by *Oprk1*), is also present in these neurons, however, remains contentious. Expression of *Pdyn* and *Tac2* in the arcuate nucleus is downregulated by estrogen, to a similar extent as *Kiss1*,[63] which suggests involvement in negative feedback.

Further interest regarding the physiology of the neurokinin B system and its effect on regulation of the reproductive axis has come from human studies that have linked loss-of-function mutations in the genes encoding neurokinin B (*TAC3*) or neurokinin B receptor (*TAC3R*) to lack of puberty and isolated hypogonadotropic hypogonadism.[67–69] In ewes, monkey and rat studies have shown stimulatory action of the neurokinin-B agonist senktide on secretion of luteinizing hormone,[65,70,71]

which occurs in a GnRH-dependent manner.[65] Similarly, studies in postmenopausal women have demonstrated a rise of hypothalamic concentrations of neurokinin B linked to gonadotropin hypersecretion caused by the absence of ovarian hormones.[34] Evidence for desensitization of neurokinin-B-induced luteinizing hormone responses has been reported for monkeys;[65] this phenomenon is reminiscent of the loss of response to kisspeptins after its continuous administration.[72] The lowering of luteinizing hormone levels after repeated infusion of senktide, however, can be rescued by Kp-10,[65] which suggests that desensitization occurs upstream of GPR54. The desensitization might account for the paradoxical inhibitory effect of senktide injections on secretion of luteinizing hormone in rodents and ewes that have undergone gonadectomy,[63,70,71,73] where previously elevated endogenous levels of neurokinin B are expected. In any event, the hypogonadotropic hypogonadal phenotype of humans with genetic inactivation of the neurokinin B pathway, together with the reported effects of neurokinin-B agonists on luteinizing hormone release under physiological conditions in different species indicate a stimulatory role for neurokinin B in the central control of the HPG axis.

The demonstration of Kiss1 and neurokinin B coexpression in the arcuate nucleus and their convergent roles in gonadotropic control have prompted analyses of the mode of interplay of these two neuropeptides. The role of dynorphin A in this system is being actively investigated as well, given the long-known function of dynorphin A as an inhibitor of GnRH and gonadotropin release in various species. Accumulating data make it tempting to propose a model in which KNDy neurons form a tightly interconnected network that substantially contributes to the generation of GnRH pulses. In this scenario, the major output signal to GnRH neurons would be kisspeptins. In turn, neurokinin B (as stimulator) and dynorphin A (as inhibitor) might reciprocally operate on KNDy neurons to fine-tune kisspeptin release. This hypothesis is at least partially supported by the following experimental evidence: expression of *Fos* is induced in Kiss1 neurons in the arcuate nucleus by central senktide injection;[71] senktide elicits secretion of luteinizing hormone in a GnRH-dependent manner;[65] desensitization to the effects of continuous neurokinin B stimulation occurs upstream of GnRH neurons;[65] substantial expression of neurokinin B receptor is detected in KNDy neurons, but apparently not in GnRH neurons, in sheep and rodents;[61] and the neurokinin-B agonist evokes the electrical activation of Kiss1 neurons, as revealed by whole-cell-current clamp recordings in *Kiss1*-Cre/GFP mice (V. M. Navarro, unpublished work). As stated in previous sections, the existence of a dense complex of neurokinin-B fibers surrounding Kiss1 cells in the arcuate nucleus provides the anatomical basis for such an autoregulatory network.[41] The fact that multiunit activity volleys, used as a surrogate marker of GnRH pulses, in goat mediobasal hypothalamus were stimulated by neurokinin B, but inhibited by dynorphin A, adds further strength to the theory.[64] Investigation is continuing of the physiological roles of this arcuate nucleus KNDy pathway in the dynamic control of GnRH neurons by different regulators, including sex steroids and metabolic cues.

Kiss1, metabolic status and fertility

Reproductive function is sensitive to various factors, including changes in metabolic status and energy reserves.[6,74] This phenomenon is especially evident at puberty onset and for reproductive capacity in females, where the size of fuel stores is crucial because of the amount of energy needed for pregnancy and lactation. Yet, reproductive function in males is also sensitive to conditions of metabolic stress.[75] Accordingly, energy imbalance owing to energy insufficiency or overweight or obesity, affects puberty, fertility or both. A threshold of body energy stores is mandatory for puberty to proceed and for the maintenance of reproductive capacity, but large energy stores can alter the timing of puberty and lead to impaired fertility as well.[8]

Although the effect of several metabolic hormones and nutritional signals on the central function of the HPG axis is well-documented,[5,75] GnRH neurons seem to lack receptors for most of those factors. For instance, GnRH neurons have no functional receptors for leptin, despite its key role in permissive signaling for puberty onset and fertility.[76] This finding suggests the existence of intermediate pathways transmitting metabolic information to this key neuron population. Evidence suggests that the hypothalamic Kiss1 system is involved, as documented by several expression and functional studies in models of metabolic stress.[8,74] Debate has, however, arisen about the nature of and the primary site or sites for the action of kisspeptins in the metabolic control of puberty and fertility, as is discussed in the following sections.

So far, most of the analyses of metabolic control of the Kiss1 system have been conducted in models of acute or persistent negative energy balance, owing to known inhibitory effects on the HPG axis that prevent puberty onset, decrease circulating concentrations of gonadotropin and sex steroids and lead to anovulation.[77,78] Undernutrition and metabolic distress causing hypogonadotropism suppress hypothalamic *Kiss1* expression and kisspeptin content. The experimental evidence supporting this contention can be summarized as follows: in pubertal rats subjected to fasting, hypothalamic *Kiss1* mRNA levels[24] and the number of immunoreactive Kiss1 neurons in the arcuate nucleus are notably decreased;[74] food deprivation for 48 h in adult female rats is associated with decreased *Kiss1* mRNA levels in the AVPV/PeN;[79] in male mice subjected to fasting, hypothalamic *Kiss1* expression is decreased as early as 12 h after food deprivation begins;[80] short-term fasting lowers hypothalamic levels of *KISS1* mRNA in monkeys;[81] chronic subnutrition during puberty reduces *Kiss1* mRNA levels in the arcuate nucleus of female rats;[82] *Kiss1* expression is decreased in the arcuate nucleus and preoptic area of ewes after chronic food restriction;[83] and conditions of metabolic stress and negative energy balance caused

by uncontrolled diabetes mellitus evoke a reduction in hypothalamic expression of *Kiss1* mRNA in rats.[84,85] The state of negative energy balance during lactation is also associated with decreased concentrations of immunoreactive kisspeptins in the arcuate nucleus and *Kiss1* mRNA levels in the AVPV/PeN and arcuate nucleus.[86,87]

Acute central injection of Kp-10 causes potent gonadotropic responses in rats subjected to fasting, despite the prevailing decrease of basal gonadotropin levels, which supports the functional relevance of suppressed kisspeptin tone in the inhibition of HPG function in the presence of energy insufficiency. Similarly, repeated injections of kisspeptins to chronically malnourished peripubertal female rats reverses puberty arrest, as is evidenced by restoration of the vaginal opening and induction of potent gonadotropic and estrogenic responses, even when caloric restriction is continued.[24] Treatment with Kp-10 also reverses the hypogonadotropic status of uncontrolled diabetic male and female rats,[84,85] and when chronically injected into the brain, ameliorated the hypogonadal state in male rats with long-term diabetes mellitus.[84] In women with hypothalamic amenorrhea, which is often associated with conditions of negative energy balance, twice-weekly administration of Kp-54 over an 8-week period was significantly associated with increased circulating gonadotropin levels.[88] When considered jointly, the expression and functional evidence indicates that Kiss1 neurons in the hypothalamus can sense changes in the metabolic status of an organism, especially in extreme conditions of energy insufficiency. In addition, they can transmit relevant information to downstream GnRH neurons to adjust reproductive maturation and function to the size of energy reserves and general metabolic conditions.

The effects of persistent overweight on the expression and function of the hypothalamic Kiss1 system has been studied much less than those of energy insufficiency. However, the available evidence does suggest that obesity perturbs the expression of *Kiss1* and is a putative mechanism for the alterations of the HPG axis commonly observed in this condition. Long-term, diet-induced obesity in C57BL6 mice was associated with very low levels of circulating testosterone. The expected increase in hypothalamic *Kiss1* mRNA levels was not seen, which suggests some degree of impairment of Kiss1 responses to key physiologic regulators in obese animals.[80] In the same vein, sustained obesity in DBA/2J mice was associated with a marked decrease in *Kiss1* mRNA levels in the arcuate nucleus and AVPV/PeN and in the number of immunoreactive Kiss1 neurons in the AVPV/PeN.[89] Our own data suggest that early-onset overweight in female rats causes acceleration of puberty and increased *Kiss1* mRNA levels in the hypothalamus, and possibly increased numbers of immunoreactive Kiss1 neurons in the AVPV/PeN.[90] Persistent conditions of energy excess do seem, therefore, to affect the hypothalamic Kiss1 system, whose nature (stimulation versus inhibition) and consequences (earlier puberty onset versus later suppression of HPG function) might depend on the timing and magnitude of overweight.

Metabolic regulators of Kiss1

Identification of effects of metabolic conditions and energy status on the Kiss1 system led to the analysis of the putative signals and mechanisms involved. Much has been learned, but the physiologic relevance and the locations of the primary sites of action of some putative metabolic modulators have been questioned. The main points of this debate are reviewed in this section.

Leptin and the hypothalamic Kiss1 system

Leptin has crucial metabolic and reproductive roles and, therefore, has been considered as a candidate for the metabolic regulation of Kiss1 neurons.[91] Data obtained in rats, mice and sheep indicate that low or absent leptin concentrations are associated with decreased expression of *Kiss1* in the hypothalamus. Neuroanatomical analyses in a leptin-deficient mouse model revealed a marked suppression of *Kiss1* mRNA levels in the arcuate nucleus[92] and decreased numbers of immunoreactive Kiss1 neurons in the AVPV/PeN.[89] Administration of leptin to leptin-deficient mice and lean ewes or to rats with uncontrolled diabetes mellitus as a model of metabolic stress significantly raised *Kiss1* mRNA levels in the hypothalamus.[83,84,92] Studies have also shown that leptin administration in women can reverse hypothalamic amenorrhea associated with persistent negative energy balance,[93] although the involvement of the Kiss1 system in this phenomenon in humans has not been proven yet. One study demonstrated that up to 40% of Kiss1 neurons in the arcuate nucleus in mice appear to have functional leptin receptors.[92] These observations strongly suggest that leptin acts on Kiss1 neurons as a way to conduct part of its permissive and stimulatory actions on the HPG axis.

This theory is challenged by the findings of two independent studies, which suggest that the mode of action of leptin on Kiss1 neurons is preferentially indirect. First, in mice without leptin receptors on *Kiss1*-expressing cells (as generated by the use of the Cre–loxP system) onset of puberty was normal.[94] The specificity of selective ablation of leptin receptors from postnatal Kiss1 neurons might, however, be compromised by the wider expression patterns of *Kiss1* within the brain and other peripheral tissues at early stages of maturation. In addition, given that, in this mouse model, leptin receptors were congenitally absent in Kiss1 cells, development of compensatory mechanisms could have occurred. Notwithstanding these possibilities, an immunohistochemical analysis showed no functional leptin receptors on GnRH or Kiss1 neurons, except for on a marginal subset of Kiss1 cells in the arcuate nucleus.[95] By contrast, functional receptors were seen on cells in the ventral premammillary nucleus and the preorbital area; the former has been purported to be an important node for transmitting the permissive effects of leptin to GnRH neurons.[94] In addition, in the same study, uncharacterized populations of neurons positive for functional leptin receptors were seen in close proximity to Kiss1 neurons in the arcuate nucleus and AVPV/PeN.[95] A proportion of the effects of leptin on *Kiss1* expression and kisspeptin concentrations in the

hypothalamus might, therefore, arise owing to transmission via intermediate pathways.[95] These pathways might also signal to GnRH neurons independently of Kiss1 circuits. The apparent discrepancy between RNA and protein expression of leptin receptors in Kiss1 neurons needs to be resolved, but may be hindered by current methodological limitations (for example, threshold of detection of immunohistochemistry), expression of alternative isoforms of leptin receptors, whose functional relevance at the hypothalamus has been documented in other settings, or both.[96]

To add further complexity to the issue, a report by True and co-workers suggests that leptin, at concentrations within the physiological range, is not the only critical signal for recovery of gonadotropin secretion and hypothalamic *Kiss1* expression when negative energy balance is being resolved.[97] They found that apparent restoration of physiological levels of leptin in female rats subjected to persistent caloric restriction or acute fasting was not associated with normalization of luteinizing hormone concentrations or *Kiss1* mRNA expression.[97] This finding suggests the contribution of additional metabolic modulators. The possibility that luteinizing hormone concentrations and *Kiss1* mRNA expression can normalize when leptin levels are at the thresholds of normal could not, however, be excluded. Moreover, the fact that high doses of leptin could overtly modulate luteinizing hormone concentrations and *Kiss1* mRNA expression in food-restricted females suggests a major regulatory role for leptin on kisspeptin expression and, hence, on gonadotropic function.

Other neuropeptide and hormonal pathways

Various metabolic hormones and neuropeptides in addition to leptin are probably involved in the regulation of the hypothalamic Kiss1 system. The existence of bidirectional interactions between Kiss1 neurons and several pathways with essential roles in the central control of food intake and energy balance has been proposed. For instance, *in vivo* studies in sheep and mice have suggested that the orexigenic factor NPY and the anorectic POMC-derived peptide α-MSH can modulate the hypothalamic expression *Kiss1* in a reciprocal manner. NPY is a stimulatory signal for *Kiss1* expression in mouse and sheep hypothalamus, whereas α-MSH inhibits *Kiss1* mRNA levels in the arcuate nucleus, but enhances them in the preoptic area, in sheep.[80,98] The stimulatory effect of NPY is apparently at odds with the reported ability of leptin to inhibit NPY expression in the arcuate nucleus,[99] which makes NPY unlikely to be the mediator of the positive effects of leptin on *Kiss1* gene expression. Expression of *Npy* mRNA was stimulated by Kp-10 in sheep hypothalamus and mouse neuronal cell lines,[83,100] whereas Kp-10 suppressed the hypothalamic expression of *POMC* in sheep.[83] The latter observations are intriguing, as initial reports showed no significant effects of central injection of kisspeptins on food intake,[24] yet NPY and α-MSH are pivotal regulators of feeding. However, neuroanatomical and electrophysiological studies in mice have shown that Kiss1 neurons form synaptic contacts with POMC neurons and that Kp-10 excited POMC neurons in a dose-dependent manner,[101] whereas kisspeptins inhibited NPY neuronal activity.[101] In another study, Kp-10 increased satiety and decrease food intake after central injection in mice.[102] Additionally, the orexigenic melanin-concentrating hormone (MCH) suppresses kisspeptin-induced stimulation of GnRH neurons.[103] Thus, Kiss1 neurons seem to interact with complex orexigenic and anorexigenic pathways that probably contribute to the reciprocal interplay between energy status and reproduction at different developmental stages.

Other putative endocrine regulators of the Kiss1 system with prominent metabolic actions include ghrelin, insulin-like growth factor 1 (IGF-1) and elements of the hypothalamic–pituitary–adrenal axis. The gut-derived hormone ghrelin, the secretion of which is deemed a signal of energy insufficiency, suppresses the hypothalamic expression of *Kiss1* mRNA.[104] This finding supports the reported inhibitory role of ghrelin on the HPG axis. By contrast, IGF-1 increased the expression of *Kiss1* mRNA in the AVPV/PeN in prepubertal female rats.[105] Blockade of the IGF-1 receptor, however, did not modify *Kiss1* levels in these nuclei in adult female rats,[106] nor did IGF-1 increase *Kiss1* mRNA expression in the murine hypothalamic cell line N6.[80] Finally, the glucocorticoid corticosterone and the neuropeptide corticotropin-releasing factor decrease the hypothalamic expression of *Kiss1*,[107] which probably contributes to the well-known suppressive effects of stress on puberty and fertility. No role for insulin in the direct control of *Kiss1* expression has yet been substantiated.

Intracellular signaling systems

Several intracellular systems are involved in the sensing of cellular energy status to match energy availability and key cell functions.[108,109] Some of these systems, which act in the brain, also operate as central metabolic gauges and transmitters for the regulatory actions of various hormonal signals, such as leptin. Thus they have important roles in energy homeostasis at the whole-body level.

One of the cellular energy sensors that has attracted attention in relation to the Kiss1 system is mTOR. In addition to its many cellular roles, mTOR signaling in some neuronal hypothalamic nuclei is thought to be important for the central control of energy homeostasis;[110,111] activation of brain mTOR activity was initially shown to inhibit food intake.[110] In the arcuate nucleus, mTOR signaling has been suggested to mediate the effects of key hormonal regulators, such as leptin, on feeding.[110] The roles and regulatory mechanisms of hypothalamic mTOR are more complex and diverse than originally reported,[112] but the initial data raised the possibility of central signaling to other neuroendocrine axes, including the HPG system.

Central mTOR signaling is thought to have a role in the transmission of the permissive and stimulatory effects of leptin on the gonadotropic axis, at least partly, via modulation of *Kiss1* expression in specific hypothalamic nuclei. This contention is supported by the following experimental evidence: activation of central

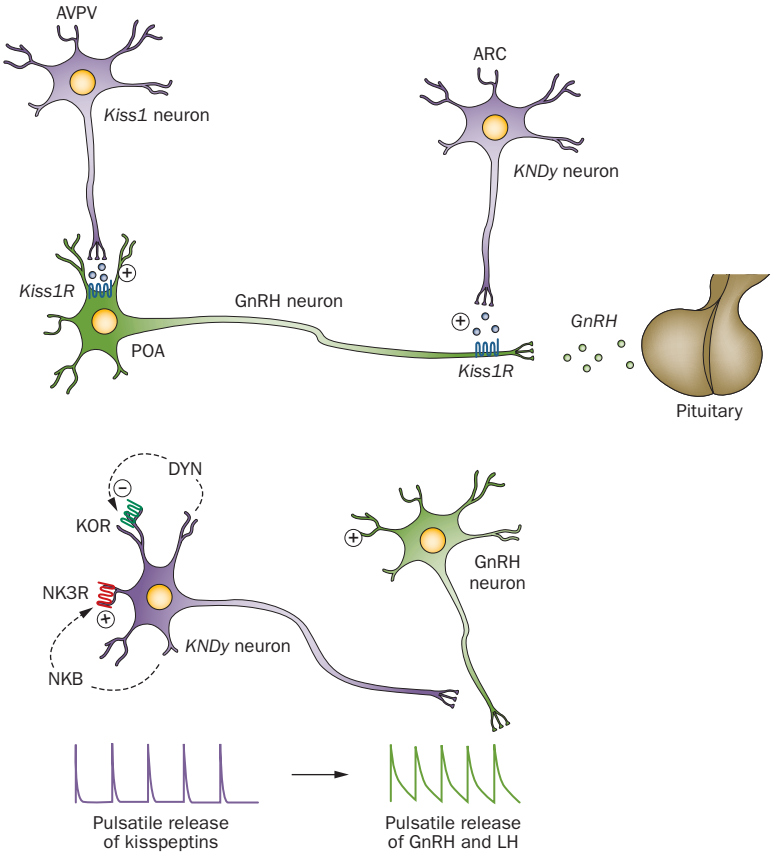

Figure 1 | A tentative model for the regulation of GnRH secretion by two distinct Kiss1 neuronal populations, located in the ARC and AVPV/PeN, as in rodents, is presented. The AVPV/PeN population is involved in positive feedback regulation by estradiol and the control of the surge-mode of gonadotropin secretion. The ARC population seems to play a pivotal role in the regulation of the tonic-mode of gonadotropin secretion and mediation of negative feedback of sex steroids. ARC Kiss1 neurons coexpress NKB and dynorphin A and, therefore, have been termed KNDy. NKB and dynorphin A are thought to operate in a reciprocal manner (stimulatory and inhibitory, respectively) as autosynaptic regulators of the kisspeptin output to GnRH neurons. Thus, they play crucial parts in the control of the pulsatile secretion of GnRH. Abbreviations: ARC, arcuate nucleus; AVPV/PeN, anteroventral periventricular nucleus/rostral periventricular nuclei; GnRH, gonadotropin-releasing hormone; KNDy, coexpressing Kiss1, NKB and dynorphin A; KOR, κ-opioid receptor; LH, luteinizing hormone; NKB, neurokinin B; NK3R, NKB receptor; POA, preoptic area.

mTOR signaling by L-leucine acutely stimulates secretion of luteinizing hormone and partly reverses the hypogonadotropic state of pubertal female rats under conditions of chronic subnutrition; blockade of central mTOR signaling by sirolimus (also known as rapamycin) decreases basal levels of luteinizing hormone without altering this hormone's responses to key activators of the gonadotropic axis such as kisspeptins; sustained blockade of central mTOR signaling disrupts the normal timing of female puberty in rats, as demonstrated by delayed vaginal opening, low ovarian and uterus weights, impaired ovarian follicular development and suppressed ovulation; the permissive effects of leptin on puberty onset in female rats are abrogated by the concomitant inhibition of mTOR signaling; and sustained blockade of central mTOR signaling markedly suppressed *Kiss1* mRNA levels in the arcuate nucleus and, to a lesser extent, the AVPV/PeN.[82] The neuroanatomical substrate for this phenomenon remains unknown. Intuitively, mTOR would operate within Kiss1 neurons to modulate their gene expression. However, a study has shown that pS6, a downstream target and marker of mTOR activity, is not detectable in Kiss1 neurons[89] and, therefore, intermediate elements might play a part. A leptin–mTOR–Kiss1 pathway has been hypothesized, which is compatible with the proposed mode of indirect action of leptin upon Kiss1 neurons.[95]

Another intracellular signaling system with a putative role in the control of reproduction involves CREB-regulated transcription coactivator 1 (encoded by *Crtc1*), which participates in the modulation of *Kiss1* gene expression.[113] This cytoplasmic transcription coactivator shuttles to the nucleus upon dephosphorylation (activation) in response to cAMP and calcium signals. *Crtc1* knockout mice exhibit obesity, as a manifestation of disturbed energy homeostasis, and infertility.[113] The mechanism for the reproductive alterations in this mouse model probably involves the impairment of intracellular signaling of leptin, given that leptin dephosphorylates and activates Crtc1 and stimulates its recruitment to the *Kiss1* gene promoter to induce transcription.[113] A leptin–Crtc1–Kiss1 pathway seems, therefore, feasible for the transmission of nutritional and metabolic information to the centers governing fertility. However, the consequences of functional inactivation of Crtc1 on mouse fertility could not be replicated,[114] and the model of leptin-induced direct Crtc1 regulation of *Kiss1* transcription does not fit with the proposed indirect mode of action of leptin in the regulation of *Kiss1* expression.[94,95]

Future kisspeptin research

Multiple areas of interest and research efforts have converged and enabled astonishing progress in the knowledge of fundamental features of the development, maturation and function of the reproductive axis. Further advances in understanding the Kiss1 system in normal and pathologic conditions seem probable. Although we cannot discuss all expected directions here, we briefly forecast potential avenues of kisspeptin research that we believe will expand in the near future.

Much excitement has emerged because of the recognition of the indispensable roles of neurokinin B and its receptor in the neuroendocrine control of reproduction in humans and probably other mammals, and their coexpression with kisspeptins in Kiss1 neurons in the arcuate nucleus. A tentative theory for the dynamic interplay between neurokinin B and kisspeptins for the fine regulation of pulsatile GnRH secretion has been developed, where neurokinin B operates as a stimulatory signal for the autoregulation of Kiss1 neuronal output in terms of kisspeptin release to stimulate GnRH neurons (Figure 1). The evidence supporting this model is convincing, but some aspects of this network remain ill-defined and require further analyses. The following features require detailed characterization: the eventual actions of neurokinin B directly on GnRH neurons and their physiological relevance, if any; the

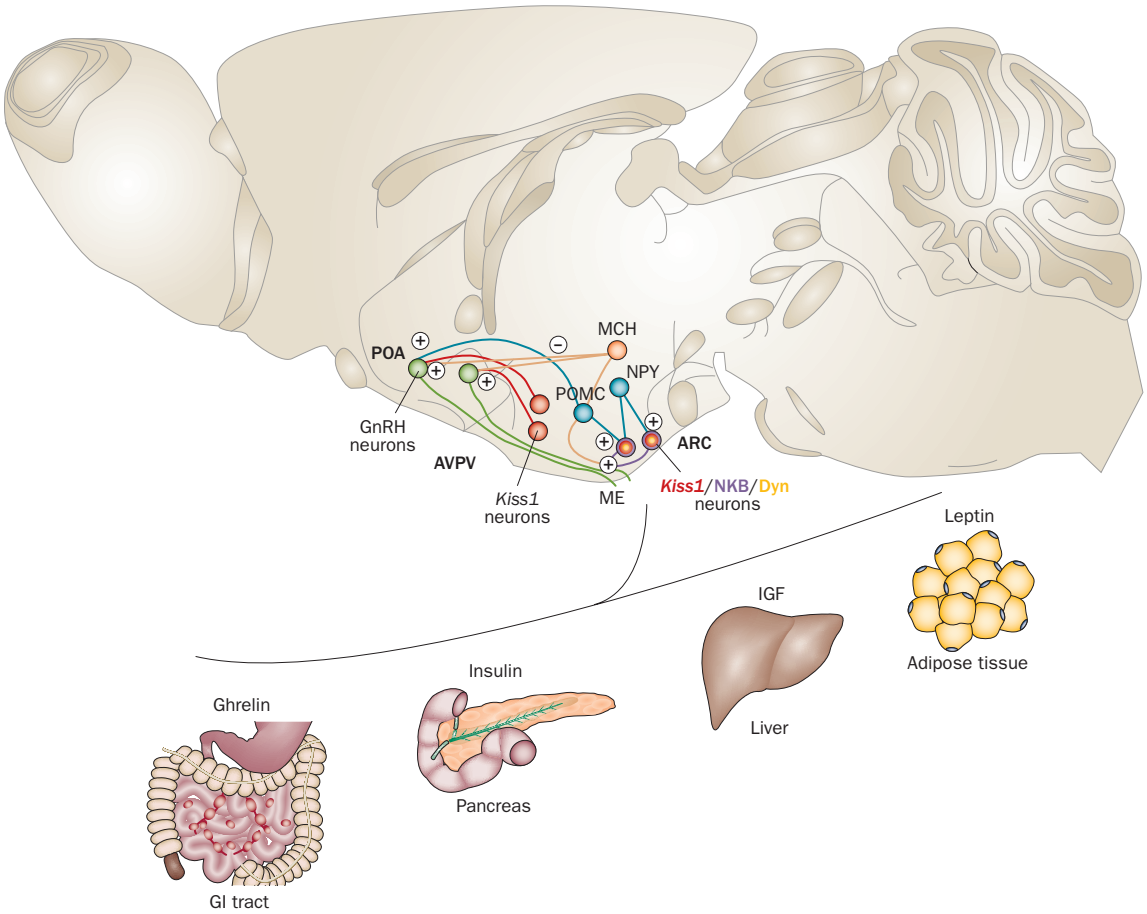

Figure 2 | Major hormonal, neuropeptide and molecular metabolic regulators of Kiss1 neurons. Some of the major putative metabolic regulators of Kiss1 neurons, involved in the matching of energy balance, metabolic status and reproduction, are presented. These regulators comprise peripheral hormones with key roles in energy and body weight homeostasis. In addition, several central neuropeptide pathways with key roles in the control of body weight and feeding modulate *Kiss1* expression, kisspeptin actions or both. These are likely to include the orexigenic NPY and MCH and the anorexigenic POMC-derived peptide α-MSH. The interplay of some of these central transmitters and kisspeptins might be bidirectional. Finally, mTOR and Crtc1 have been suggested as putative molecular mediators for at least some of the effects of key metabolic regulators of *Kiss1* expression, such as leptin, but their roles are not yet clear; for sake of concision, these are not depicted in the scheme. Abbreviations: ARC, arcuate nucleus; AVPV/PeN, anteroventral periventricular nucleus/rostral periventricular nuclei; GI, gastrointestinal; GnRH, gonadotropin-releasing hormone; IGF, insulin-like growth factor; KNDy, coexpressing Kiss1, NKB and dynorphin A; ME, median eminence; POA, preoptic area.

effects of neurokinin-B agonists and antagonists on the firing patterns of Kiss1 neurons; the putative roles of neurokinin-B signaling in mediating negative, and potentially positive, feedback actions of sex steroids; and the actual roles of neurokinin B as a potential modulator of the timing of puberty. In addition, the mechanism behind the disparate effects of neurokinin B agonists on secretion of luteinizing hormone according to gonadal status merits further investigation. For instance, in female rodents, neurokinin-B agonists potently stimulate release of luteinizing hormone in intact, cyclic female rats, but it inhibits secretion in animals that have undergone gonadectomy. Finally, the phenotype of neurokinin-B neurons in the arcuate nucleus (for example, coexpression with Kiss1) and the set of their putative regulators, from sex steroids to metabolic factors, await clarification. What the molecular determinants are for the selective coexpression of neurokinin B in arcuate nucleus but not AVPV/PeN Kiss1 neurons would be useful to know.

Substantial efforts have been devoted to revealing the interactions between kisspeptins and other central neurotransmitters involved in the control of GnRH secretion. In addition to metabolic neuropeptides, such as NPY, α-MSH and MCH, and the cotransmitters neurokinin B and dynorphin A, classic transmitters have been studied. Evidence for kisspeptin-dependent and kisspeptin-independent effects of glutamatergic neurotransmission on GnRH secretion, via the NMDA receptor, has been presented.[115] Similarly, electrophysiological studies suggest kisspeptin actions on GnRH neurons derive partly from their capacity to increase GABA and NMDA transmission onto this neuron population; this phenomenon is estradiol-dependent.[116]

This area of kisspeptin research is expected to remain active, as it should enable mapping of the hierarchical roles and elucidate the relative functional relevance of various direct and indirect afferents to GnRH neurons, including kisspeptin inputs themselves. The suggestion of indirect modes of action by key regulators, such as leptin, on Kiss1 neurons has fueled interest in this area.

Refinement of animal models to enable analysis of different facets of the physiology of the Kiss1 system would be beneficial. So far, genetically modified models for the study of kisspeptin signaling have been restricted to *Kiss1* and *Gpr54* null mice.[18] In the past few months though, *Kiss1*-driven Cre mouse lines have been described.[53,94] The power of this model stems not only from the possibility to directly visualize and analyze Kiss1 neurons (if coupled to the GFP marker), but also to selectively ablate specific targets within Kiss1 cells, by conventional Cre–loxP methods.[53,94] Developments are anticipated to involve elimination of various regulatory pathways in Kiss1 cells, the generation of related models (for example, the generation of *Kiss1*-floxed or *Gpr54*-floxed mice that enable selective elimination of these genes in specific cellular targets by promoter-driven Cre expression) or both. The use of *Kiss1*-Cre/GFP models developed with Cre/GFP constructs or through breeding to GFP-floxed lines to selectively target adult populations of Kiss1 neurons in the arcuate nucleus and AVPV/PeN might, however, be hampered by the wider patterns of expression of the *Kiss1* gene at different developmental stages. Furthermore, the different strategies for *Kiss1*-directed Cre expression (that is, conventional transgenesis versus knock-in approaches) might affect the selective targeting of Kiss1 cells. In this context, the generation of tools for selective overexpression or elimination of *Kiss1*, or eventually *Gpr54*, in specific developmental windows, hypothalamic nuclei or both, would enable the functional dissection of the roles of the major populations of Kiss1 neurons in the control of the HPG axis at different stages of maturation.

Mayer and Boehm[117] showed that ablation of Kiss1 neurons at very early developmental stages was apparently compatible with normal puberty progression and fertility in female mice. Mice with analogous elimination of Gpr54 cells, which include GnRH neurons, displayed preserved fertility. These phenotypes are particularly striking, given the well-documented reproductive effect of congenital inactivation of either *Kiss1* or *Gpr54*. Different phenomena might contribute to these apparent contradictory findings, for instance, the possibility that plastic developmental compensatory mechanisms arose after complete ablation of these key reproductive cell populations at very early stages of development; compensation might not occur after selective elimination of a single, essential gene or factor, such as *Kiss1*. In addition, a subset of cells expressing Kiss1 (or Gpr54) in these models may have escaped ablation[117] and might be sufficient to drive puberty and fertility, as previously documented for GnRH neurons.[118] In the same study though, timely ablation of Kiss1 neurons during the prepubertal period (postnatal day 20) resulted in overt reproductive failure in adulthood,[117] which underscores the essential roles for kisspeptin pathways in the central regulation of puberty and reproduction.

After initial characterization of the putative regulatory roles of several metabolic hormones and neuropeptides (Figure 2), studies are needed to detail the whole set of signals responsible for the control of the Kiss1 system by nutritional and metabolic cues. Besides the identification of novel regulatory factors, these studies will probably focus on the modes of action of these signals in order to determine whether they operate directly on Kiss1 neurons or indirectly via intermediate pathways, as is suggested for leptin. Similarly, the intracellular mechanisms involved in the regulation of *Kiss1* expression should be further evaluated. The potential roles of kisspeptins as modulators of the major neuropeptide pathways involved in the central control of food intake and energy balance, such as NPY and POMC neurons in the arcuate nucleus, merit further analysis. Lastly, although the effects of energy insufficiency on *Kiss1* expression and kisspeptin concentrations are well-characterized, the consequences of persistent obesity on the expression and function of this system remain ill-defined. Of particular translational relevance will be whether early-onset obesity causes changes in the maturation of hypothalamic Kiss1 circuits that could be associated to perturbation of the timing of puberty. Similarly, further characterization of the inhibitory impact of long-term obesity on hypothalamic *Kiss1* expression and kisspeptin concentrations might help to define the neuroendocrine basis for the state of hypogonadotropic hypogonadism that is commonly associated with this condition.

Conclusions

Kisspeptins act as gatekeepers of the maturation and function of the HPG axis. Kiss1 neurons are major upstream regulators of GnRH neurons and conduits for the modulatory effects of numerous central and peripheral signals that influence reproduction. In this context, Kiss1 neurons have an important role in the pathways by which metabolic conditions and energy homeostasis modulate puberty onset and fertility. The function of Kiss1 neurons in the arcuate nucleus is (auto)regulated by neurokinin B, which is essential for human reproduction. The Kiss1 system has not only a crucial role in the central control of reproduction but also pathophysiological potential and might yield therapeutic targets that will be of paramount importance in reproductive endocrinology and medicine.

Review criteria

We searched PubMed in February to June 2011 for original articles and (seminal) reviews focused mainly on the reproductive and neuroendocrine actions of kisspeptins, with special emphasis on their roles as mediators for the metabolic control of puberty and fertility. The search terms were "kisspeptin", "GPR54", "Kiss1", "KISS1", "metastin" and "Kiss1R".

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Author contributions

Both authors contributed equally to all aspects of the article.