



Review

New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function

Juan Roa ^{a,c}, Enrique Aguilar ^{a,c}, Carlos Dieguez ^{b,c}, Leonor Pinilla ^{a,c},
Manuel Tena-Sempere ^{a,c,*}

^a *Physiology Section, Department of Cell Biology, Physiology and Immunology, Faculty of Medicine, University of Córdoba, Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain*

^b *Department of Physiology, University of Santiago de Compostela, 15705 Santiago de Compostela, Spain*

^c *CIBER (CB06/03) Fisiopatología de la Obesidad y Nutrición, Instituto Salud Carlos III, 28029 Madrid, Spain*

Abstract

Identification, in late 2003, of inactivating mutations of the G protein-coupled receptor GPR54 as causative factor for absence of puberty and hypogonadotropic hypogonadism in humans and mice was a major breakthrough in modern Neuroendocrinology, and drew considerable interest on the characterization of the roles of this receptor and its ligands (kisspeptins, encoded by the *KiSS-1* gene) in the physiological control of essential facets of reproduction. After 3 years of intense research activity, kisspeptins are universally recognized as essential activators of the gonadotropic axis, with key roles in puberty onset and the control of gonadotropin secretion. While these fundamental functions are now well settled, novel aspects of kisspeptin/GPR54 physiology have emerged, including their involvement in the neuroendocrine control of ovulation and the metabolic gating of reproductive function. In addition, the ‘comparative endocrinology’ of this system has begun to be explored recently. These facets of kisspeptin/GPR54 function, as fundamental gatekeepers of reproduction, will be comprehensively reviewed herein.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Kisspeptin; *KiSS-1*; GPR54; Gonadotropins; Gonadotropin-releasing hormone (GnRH); Puberty; Energy balance; Leptin; Ovulation; Hypogonadism

1. Introduction

Reproductive function, as essential for the perpetuation of species, is subjected to the precise control of a complex network of regulatory signals and influenced by a wide spectrum of endogenous and external conditions [27,28,57,84,113,118,122]. In addition, development and maturation of the different elements of the reproductive system is an exquisitely timed phenomenon; a continuum of differentiating events that spans from early fetal life to puberty [80]. Accordingly, the functionality of the reproductive system undergoes dramatic changes throughout post-natal development, from the neonatal and infantile

periods to puberty onset, adulthood and, eventually, senescence [28,80,118]. Such intricate functional and developmental features evidence the multi-faceted nature of the systems responsible for attainment and maintenance of reproductive capacity in mammals and other vertebrates.

Although significant progress in the field during the last decades had (apparently) allowed to identify the major players of the reproductive axis, in late 2003, the unsuspected demonstration of deletions and inactivating mutations of the gene encoding the G protein-coupled receptor GPR54 in patients suffering familial and sporadic forms of hypogonadotropic hypogonadism (HH) unveiled the reproductive ‘dimension’ of this receptor and its putative ligands, the kisspeptins—encoded by the *KiSS-1* gene [22,104]. This pioneering observation opened up a new era in our understanding of the neuroendocrine mecha-

* Corresponding author. Fax: +34 957 218288.

E-mail address: filtesem@uco.es (M. Tena-Sempere).

nisms for the control of reproduction. Indeed, the attention drawn by this ‘novel’ ligand–receptor system among reproductive physiologists has been enormous, as illustrated by the large number of research articles and reviews appeared on this particular topic during the last 3 years (>125 in PubMed database). These studies have now defined the fundamental role of kisspeptins and GPR54 in the control of key aspects of reproduction, from puberty onset to regulation of gonadotropin secretion in adulthood; findings that are collectively regarded as one of the most significant breakthroughs in Reproductive Neuroendocrinology since the isolation of gonadotropin-releasing hormone (GnRH) in early 1970s [62]. However, after such a thorough, initial characterization, new *frontiers* in kisspeptin physiology (as indispensable gatekeeper of reproduction) can be now foreseen. In the present article we will provide a comprehensive review of the most salient features of the so-called KiSS-1/GPR54 system, with special emphasis on its recently recognized functions, such as in the neuroendocrine control of ovulation and the metabolic regulation of fertility, as well as other emergent areas of kisspeptin physiology.

2. Neuroendocrinology of reproduction: the hypothalamic-pituitary-gonadal axis

The myriad of regulatory signals that participate in the dynamic control of reproductive function impinge at one or different levels of a complex neuroendocrine system which is organized into three major levels of integration: the hypothalamus, the pituitary and the gonads. Such a hypothalamic-pituitary gonadal (HPG) axis, also termed gonadotropic axis, is primarily defined by the interplay of three major groups of factors (ligands and their receptors), which have been the subject of intense investigation during the past decades [28,118]. These are (i) the hypothalamic decapeptide *gonadotropin-releasing hormone* (GnRH), which selectively operates on pituitary gonadotrops to activate the episodic secretion of (ii) the *gonadotropins*, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn activate somatic cells in the gonads to induce the synthesis and release of (iii) *gonadal steroid and peptide hormones*. In addition, pituitary gonadotropins are indispensable for qualitatively and quantitatively normal gametogenesis and fertility, in both males and females [49]. Although a detail description of the major functional characteristics of this neuroendocrine system can be found elsewhere [28,118], for the purpose of present review, we highlight below some of most salient functional features of the HPG axis in mammals:

- The major hierarchical element of the HPG axis is GnRH; neurons in the forebrain expressing this neuropeptide being the target of multiple central and peripheral modulators, and serving a key role as final output pathway for the regulation of down-stream elements of the axis [39].

- GnRH neurons are controlled by a complex network of neuropeptides and transmitters (*central* regulators), of excitatory (e.g. glutamate, noradrenalin) and inhibitory (e.g. γ -aminobutyric acid—GABA, endogenous opioid peptides—EOP) nature [80]. Regulation of GnRH neurons involves not only trans-synaptic signals, but also glial-to-neuron inputs, which include growth factors and, likely, glutamate [78,79].
- The major hormonal elements of the HPG axis are functionally connected through feed-forward and feedback regulatory loops; gonadal steroids serving a predominant inhibitory role in terms of control of GnRH and gonadotropin secretion [118], except for the positive feedback effects of estrogen (in the presence of activated progesterone receptors) during pre-ovulatory period in the cyclic female [55].
- In addition to gonadal steroids and peptides, a wide array of *peripheral* signals participate also in the tuning of the HPG axis. Among those, energy reserves and the metabolic status of the organism are known to play key roles in the control of reproductive function, mainly through the actions of hormones originating from the adipose tissue, gastrointestinal tract and pancreas [27].
- The functionality of the HPG axis undergoes dramatic changes during development, with quiescent (humans) to decreased (rodents) activity during late infancy and juvenile periods and full activation of neurosecretory activity of the GnRH/gonadotropin axis at puberty [78,80]. In addition, the female HPG axis is subjected to functional changes along the ovarian cycle, pregnancy and lactation [101,113].

According to those major functional features, the overall consensus existed that GnRH neurons in the forebrain would operate as the major site for the integration of afferent (regulatory) signals from diverse brain areas and the periphery, dictating either the activation or eventual inactivation (in situations of suppressed GnRH secretion) of the reproductive axis in different physiological states and pathological conditions. While this general contention remains (substantially) valid, such a *GnRH-centric* conception of the reproductive system has been partially challenged in recent years by the identification of the indispensable roles of kisspeptins and GPR54 in the control of GnRH neurons and, hence, of key facets of reproductive function. Indeed, KiSS-1 neurons at the hypothalamus (whose existence remained neglected until recently) are now recognized as a fundamental element in the HPG axis, involved in the reception and integration of essential regulatory signals, such as gonadal steroids, metabolic factors and photo-periodic cues, among others. Those aspects will be discussed in detail in the following sections.

3. The KiSS-1/GPR54 system

Although the reproductive dimension of kisspeptins and GPR54 has attracted most of the attention devoted to this

ligand–receptor pair recently [32,102,115], identification of the elements of this system preceded in several years the discovery of their fundamental roles in reproduction. In this section, we will provide basic information about the *history* of identification and nomenclature of the components of this system. In addition, a brief overview of the biological functions initially assigned to KiSS-1 and GPR54, and those not directly related with their role in the neuroendocrine control of the gonadotropic axis, is also included.

3.1. KiSS-1, kisspeptins and GPR54

As indicated above, the KiSS-1/GPR54 system is composed by a number of ligands (peptides encoded by the KiSS-1 gene) with ability to bind and activate the G protein-coupled receptor GPR54. The first element of the system to be recognized was KiSS-1, which was identified in 1996 as mRNA transcript selectively over-expressed in melanoma cell lines with low metastatic capacity [52,53]. This finding was followed in 1998 by the cloning and chromosomal localization of KiSS-1 gene, then regarded as metastasis-suppressor, in the human [127]. In a totally unrelated context, in 1999, GPR54 was cloned in the rat as an orphan receptor with a significant sequence similarity (>40%) with the transmembrane regions of galanin receptors [51]. Subsequently, the human ortholog of GPR54 was identified, and named AXOR12 or hOT7T175 [67,77]. However, it was not until 2001 when the connection between KiSS-1 and GPR54 was first established. This came with the characterization of the major product of the KiSS-1 gene, a 54 amino acid peptide named metastin by virtue of its ability to inhibit pulmonary metastasis of melanoma cells *in vivo*. Metastin was shown to act via GPR54/hOT7T175 [77], which has been referred also as KiSS-1 receptor [30]. This original finding was soon confirmed by two additional studies [48,67], which added further depth to the knowledge of this system: the KiSS-1 gene encoded a number

of structurally-related peptides, which derive from the differential proteolytic processing of a common precursor of 145 amino acids, containing a putative 19 amino acid signal sequence, two potential dibasic cleavage sites (at amino acids 57 and 67) and one putative site for terminal cleavage and amidation (at amino acids 121–124) [48,67,77]. The family of KiSS-1 peptides included not only metastin (also termed kisspeptin-54, which appeared to be abundantly expressed in the placenta), but also shorter fragments of the C-terminal region of metastin molecule, such as kisspeptin-14, kisspeptin-13 and kisspeptin-10, with 14, 13 and 10 amino acid length, respectively [48]. In fact, although ‘metastin’ was initially coined to define the major peptide product of the KiSS-1 gene [77], with the recognition of their reproductive role, the term ‘kisspeptins’ has become widely used to collectively define this family of peptides, which show an Arg-Phe-NH₂ motif at the C-terminus, distinctive of the RF-amide peptide super-family. A summary of the most salient structural features of the KiSS-1 precursor and different kisspeptins is provided in Fig. 1.

3.2. Functional biology of the KiSS-1/GPR54 system: the pre-reproductive era

As indicated in previous sections, KiSS-1 was originally regarded as a metastasis-suppressor gene and, accordingly, initial functional analyses of metastin and other kisspeptins, as well as of GPR54 (once it was defined as KiSS-1 receptor), were focused on their putative role in the control of tumor progression and dissemination. Detailed description of those studies is clearly beyond the scope of this review, and can be found elsewhere [37]. In brief, functional analyses demonstrated that metastin and other kisspeptins are able to suppress metastasis in several solid tumors, such as melanoma, breast cancer, papillary thyroid carcinoma, pancreatic cancer cells and ovarian carcinoma [45,53,54,59,77]. Moreover, KiSS-1 protein secretion has

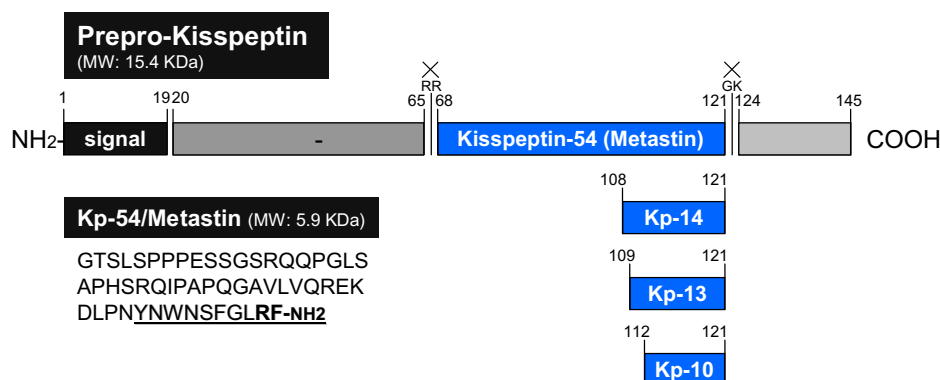


Fig. 1. Structure of human prepro-kisspeptin (encoded by the KiSS-1 gene), and major peptide products (kisspeptins) generated by differential cleavage from the common precursor. Prepro-kisspeptin is a 145 amino acid protein which contains a 19 amino acid signal peptide and a central 54 amino acid region, flanked by two consensus cleavage sites (denoted by X). Proteolytic processing of prepro-kisspeptin generates metastin or kisspeptin-54. Further cleavage of metastin gives rise to kisspeptins of lower molecular weight: kisspeptin-14 (Kp-14), Kp-13 and Kp-10. The complete amino acid sequence human kisspeptin-54 is shown, where the consensus C-terminal RFamide motif is indicated in bold. In addition, the conserved sequence corresponding to Kp-10, the shortest peptide fragment of metastin with ability to activate GPR54, is underlined. Taken from [115], with modifications.

been recently shown to suppress multiple organ metastasis and to improve survival in athymic mice injected with C8161.9 human melanoma cells [68]. In addition, down-regulation of KiSS-1 gene expression was identified as bad prognosis index for metastasis in a number of tumors, such as melanomas, as well as gastric, bladder and esophageal squamous cell carcinomas [23,42,53,99]. Altogether, the above molecular and clinical evidence strongly suggested that the KiSS-1/GPR54 system might play a role in the limitation of metastasis and tumor progression [37]; a phenomenon with obvious therapeutic implications. However, whether KiSS-1 is a universal suppressor of cancer metastasis is now under debate, as some recent studies in human breast cancer and hepatocellular carcinoma evidenced that, at least in those tumors, over-expression of KiSS-1 resulted in a more aggressive phenotype and worsened prognosis [58,100].

Initial functional analyses of the KiSS-1 system included not only studies on the effects of kisspeptins on tumor metastasis, but also evaluation of the signaling properties of GPR54, as their cognate receptor, using heterologous cell systems (CHO K1 cells stably expressing GPR54). These analyses demonstrated that (i) all kisspeptins are able to bind and activate GPR54, with kisspeptin-10 having maximal activity at the receptor level [48]; and (ii) upon receptor activation, the major intracellular signaling pathways recruited by GPR54 include activation of phospholipase C and PIP₂ hydrolysis, followed by accumulation of inositol-(1,4,5)-triphosphate, Ca²⁺ mobilization, arachidonic acid release, and phosphorylation of ERK1/2 and p38 MAP kinases [48]. Very recently, by the use of a pharmacological antagonist approach, we have provided evidence for the relevance of (most of) those pathways in conveying the biological actions of kisspeptin-10 in a physiologically-relevant target tissue, such as the hypothalamus [10].

Although its facet as potential ‘metastasis-suppressor’ drew most of the attention on this system before late 2003, the expression of KiSS-1 and GPR54 genes in an array of different non-tumoral tissues, as initially demonstrated in the human in 2001 [67,77], strongly suggested that this signaling system is provided with additional biological roles other than inhibition of tumor spread. This contention has now been substantiated, not only by the characterization of its key role in the neuroendocrine control of reproduction, but also by the identification of the expression and potential functions of KiSS-1 and GPR54 in different tissues and biological systems. Some of the experimental evidence gleaned in recent years supporting additional roles of the KiSS-1 system, other than the central control of gonadotropic axis, is summarized in the following points:

- Prominent expression of KiSS-1 and GPR54 has been demonstrated in human placenta [67,77], with peak expression of KiSS-1 gene in the first trimester (period of maximal invasiveness) and a distinctive pattern of distribution: kisspeptins are predominantly found in the

syncytiotrophoblast, whereas GPR54 is located also in the extravillous trophoblast [40]. In addition, expression of KiSS-1 and its receptor has been identified in trophoblast giant cells of rat placenta [121].

- During human pregnancy, circulating metastin levels have been reported to dramatically increase; ~7000-fold increase over non-pregnant concentrations [41]. Moreover, plasma kisspeptin levels are raised in patients with gestational trophoblastic neoplasia and fall during chemotherapy treatment [25]. In addition, functional analyses have demonstrated that kisspeptin-10 is able to inhibit trophoblast invasion [5]. The physiological relevance of this phenomenon, however, is questioned by the lack of gross abnormalities in placental formation and/or function observed in humans and mice carrying null mutations of GPR54 gene [18,83].
- Expression of KiSS-1 and GPR54 genes has been demonstrated in human and mouse pancreatic islets, where kisspeptin and GPR54 immunoreactivity was demonstrated in both α and β cells. Kisspeptin-10 stimulated glucose-induced insulin secretion by pancreatic islets without affecting glucagon release [38].
- Expression of kisspeptins and GPR54 has been recently reported in aorta, coronary artery and umbilical vein, and kisspeptins have been demonstrated as vasoconstrictors in humans, thus suggesting a putative role in the control of the cardiovascular system [61].
- Initial analyses demonstrated the expression of KiSS-1 mRNA in the human brain, with a scattered distribution throughout the central nervous system, including the basal ganglia and the hypothalamus. In addition, GPR54 gene expression was also observed in the spinal cord and different human brain areas (including hypothalamus, basal ganglia, amygdala, substantia nigra and hippocampus) [67,77]. Those findings were later confirmed in the rat [121] and mouse, where KiSS-1 mRNA expressing cells were observed in the anterodorsal preoptic nucleus, the medial amygdala and the bed nucleus of the stria terminalis, as well as at different levels along the rostral–caudal extent of the hypothalamus [33].
- KiSS-1 peptides have been also found in the brain, and initial studies demonstrated the presence of metastin-like immunoreactivity throughout the CNS, with the strongest signals being detected in the dorsomedial hypothalamic nucleus, ventromedial nucleus and arcuate nucleus (ARC) within the hypothalamus, the nucleus of the solitary tract and the caudal ventrolateral medulla. In addition, immunoreactive fibers for metastin were reported in different areas of the telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon [6]. Overall, this scattered anatomical distribution was taken as suggestive of potential roles of kisspeptins in the central control of nociception and visceral regulation [6]; yet, it is noticeable that the specificity of antiserum used in the above studies needs to be fully confirmed. Very recently, the hypothalamic distri-

bution of kisspeptin immunoreactivity has been reassessed in the mouse (by using a highly specific antiserum), with discernible kisspeptin neurons being localized not only at the dorsomedial and ARC nuclei but also (very prominently) at the anteroventral periventricular nucleus [17] (see below).

- Although functional evidence for their effects at the CNS (other than the control of the HPG axis) remains scarce, kisspeptins have been shown to up-regulate excitatory synaptic transmission in hippocampal dentate granule cells [2], and pharmacological data indicated that KiSS-1 peptides may participate in the regulation of certain neuroendocrine functions, such as oxytocin release [48].

Altogether, the above data are suggestive of multiple roles of kisspeptins and GPR54 in the control of diverse (and relevant) biological functions, from placental invasion and vascular tone to central and peripheral visceral regulation. It has to be stressed, however, that such ‘additional’ functions of the KiSS-1 system remain, in general, scarcely studied, and their potential physiological relevance is yet to be fully determined.

4. The indispensable role of KiSS-1/GPR54 system in reproduction

Despite it being now regarded as a fundamental player in the reproductive brain, the role of kisspeptins and GPR54 in the control of puberty and fertility remained completely unnoticed until late 2003, when de Roux et al. [22] and Seminara and co-workers [104] independently reported the presence of deletions and inactivating mutations of GPR54 in patients suffering idiopathic hypogonadotropic hypogonadism (IHH); a syndrome characterized by delayed or absent pubertal development secondary to gonadotropin deficiency [7]. At that time, the molecular basis of HH, which is now recognized as a rather heterogeneous condition, had begun to be unraveled by the identification of loss-of-function mutations in a number of genes, such as Kall1, fibroblast growth factor receptor or GnRH receptor, in patients with hypogonadism of central origin [4,43]. Although they being rare even within the group of normosmic IHH, the causative role of null mutations of GPR54 has been now fully confirmed by a number of independent reports [14,50,83,105,120]. A schematic presentation of the known mutations of GPR54 gene is depicted in Fig. 2. As functional analyses of the mutated receptors has been also provided, those studies helped not only to better define this monogenic form of HH (whose overall frequency is lower than that of GnRH receptor mutations in normosmic HH [14]), but to initiate also the ‘dissection’ of some of the critical regions for GPR54 function; an aspect which remains to date superficially studied. Further supporting the indispensable role of GPR54 in mammalian fertility, mice carrying null mutations of GPR54 gene were also generated in late 2003, and were reported to show a reproductive phenotype analogous to that of humans suffering

GPR54 inactivation [30,104]. Notably, the reproductive consequences of genetic inactivation of KiSS-1 *in vivo* have been very recently reported [21]; KiSS-1 knock-out mice being a virtual phenocopy of GPR54 mutants. These observations stress further the essential roles of KiSS-1/GPR54 signaling in the control of gonadotropic axis and provide direct proof that kisspeptins are the physiological ligands of GPR54 [21].

4.1. The KiSS-1/GPR54 system and the reproductive brain

In a paradigmatic example of bedside-to-bench research, identification of the reproductive ‘dimension’ of GPR54 by means of genetic studies in humans immediately boosted an extraordinary interest among reproductive biologists, who aimed to characterize the major functional features of this previously unsuspected, essential element of the neuroendocrine network controlling reproduction, mainly by the use of animal models. As first step, detailed analyses on the pattern of expression of KiSS-1 (and to a lesser extent GPR54) gene within the hypothalamus, as major site for the central regulation of the gonadotropic axis, were undertaken. Thus, initial studies in the mouse unraveled the presence of cells expressing KiSS-1 mRNA at different levels throughout the rostral–caudal extent of the hypothalamus, with prominent accumulation of KiSS-1 expressing neurons (hereafter, KiSS-1 neurons) in the anteroventral periventricular nucleus (AVPV), the periventricular nucleus (PeN) and the arcuate nucleus (ARC) [33]; areas that had been previously recognized to play essential roles in the neuroendocrine control of gonadotropin secretion. Notably, this pattern of distribution of KiSS-1 mRNA within the hypothalamus, with predominant signals at the AVPV and ARC, has been repeatedly confirmed in rodents [36,46,110,111]. To our knowledge, analogous studies on the pattern of expression of GPR54 within different hypothalamic areas have not been so far reported. Yet, GPR54 mRNA expression has been detected in GnRH neurons in the rat [44].

The above RNA studies have been recently complemented by detailed analyses on the distribution of kisspeptin immunoreactivity (IR) within the hypothalamus, which identified three major neuronal populations (cell bodies positive for kisspeptin-IR): the largest one, defined by a continuum of kisspeptin neurons extending from the AVPV to the preoptic PeN, as well as two smaller populations at the ARC and the dorsomedial nucleus [17]. In addition, immunohistochemical studies also identified a dense plexus of kisspeptin-IR fibers around the ARC, with additional kisspeptin staining in fibers within the AVPV and PeN [17]. Overall, there appears to be a considerable degree of overlapping between RNA and protein data. Yet, some divergences, such as the lack of detection of KiSS-1 mRNA at the DMN, are noticed and remain to be fully explained. To our knowledge, no studies on the pattern of distribution of GPR54-IR within the hypothalamus have been so far published; a fact that is likely due to

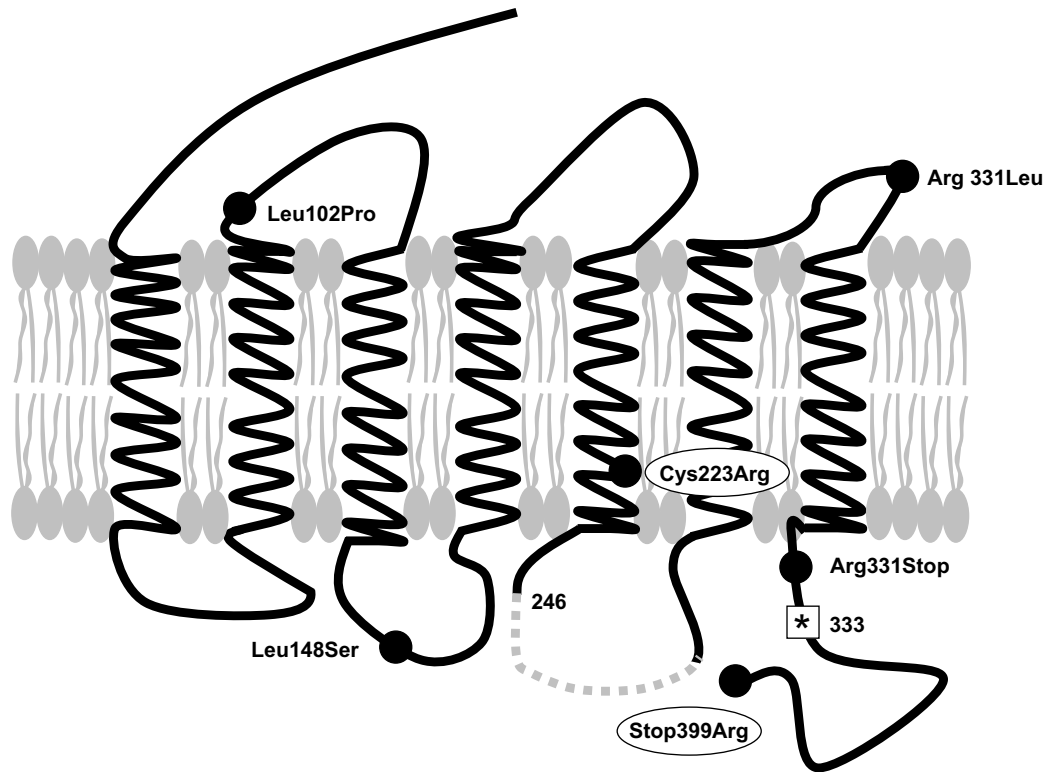


Fig. 2. Schematic representation of deletions and inactivating mutations of GPR54 gene reported so far in humans suffering hypogonadotropic hypogonadism. Inactivating mutations are indicated as *black dots*, with reference to the reported amino acid substitution. In addition, the location of the predicted deletion reported by de Roux et al. [22] is marked as *grey dots*. Finally, the position of the predicted insertion (1001_1002insC), which results in a shift of the open reading frame, as reported by Lanfranco et al. is denoted by an *asterisk*. Adapted from [120], with substantial modifications.

the lack of highly sensitive and specific antisera for its immunohistochemical detection in brain tissue.

4.2. The *KiSS-1* system and gonadotropin secretion: stimulatory effects and negative feedback

In addition to distribution analyses, one of the facets of *KiSS-1*/GPR54 physiology that was initially studied in detail was the ability of kisspeptins to regulate gonadotropin secretion, and their potential mechanism(s) of action. Thus, within 12 months from the publication of the original studies of GPR54 inactivation in HH [22,104], at least four groups independently reported the potent stimulatory effect of kisspeptin-10 and metastin on LH secretion in the rat and mouse [33,60,69,123]. Those initial studies were soon confirmed and extended to other relevant species, such as the sheep, the monkey and (importantly) the human [24,62,63,106]. In addition, detailed pharmacological analyses demonstrated that kisspeptins are able to elicit LH secretion at different stages of postnatal development (including the infantile and juvenile period in the rat and mouse) [10,36], and via different routes of administration (intracerebral—i.c.v. and intrahypothalamic- and systemic-intravenous, intraperitoneal and subcutaneous) [60,69,71,86,126]. Overall, one of the most remarkable features of the above studies was the protracted duration

(>3-h) of LH secretory responses after central and peripheral administration of kisspeptin-10 [71,126], as well as the range of sensitivity to kisspeptin, since doses of kisspeptin-10 between 100 fmol/1pmol (central administration), and ~0.5 µg/kg (peripheral administration), were sufficient to evoke robust LH secretory discharges in the rat and monkey [33,71,89,126]. Comparative meta-analysis of these data and previously published results on other well-known LH secretagogues (such as glutamate and galanin-like peptide—GALP) strongly suggests that, except for GnRH itself, kisspeptins are likely (one of) the most potent stimulators of LH secretion known so far in mammals. For instance, in protocols of central administration in rats [13,87], threshold doses for LH stimulation were in the nanomolar range for the agonist of ionotropic glutamate receptors, AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid), and GALP; i.e., ~three orders of magnitude higher than for kisspeptin [33,71]. Similarly, peripheral administration of the agonist of glutamate receptors, *N*-methyl-aspartic acid (NMA), to adult rats evoked significant LH pulses only after injection of 20 mg/kg (equivalent to 133 µmol/kg BW) [90]; a dose five orders of magnitude higher than the lowest effective intravenous dose of kisspeptin-10 [126]. Of note, despite preserved responsiveness to GnRH, GPR54 null mice were unable to respond to kisspeptin-14 in terms of LH secretion, suggesting that

the gonadotropin-releasing effects of kisspeptins are (solely) mediated via GPR54 [63].

In addition, detailed pharmacological studies in the rat have demonstrated also the ability of kisspeptins to stimulate FSH secretion. Of note, however, some interesting differences were noticed in the patterns of hormonal responses to kisspeptin between both gonadotropins. Thus, in adult male rats, FSH secretion induced by intracerebral administration of kisspeptin-10 was somewhat delayed (from 30-min onwards) as compared to the rapid LH responses (within 5–15-min) [70]. Moreover, FSH release *in vivo* appeared to be ~200-fold less sensitive to the stimulatory effect of kisspeptin than LH (EC₅₀: ~2 and 400 pmol for LH and FSH, respectively—see Fig. 3) [70]. The mechanisms for such divergence remain to be unfolded, but they might involve (i) differences in the pattern of secretion between gonadotropins, with FSH secretion being more constitutive than that of LH; (ii) differences in the effects of kisspeptins on the pattern of GnRH release, with the predominant activation of a profile of high frequency GnRH peaks which favors LH secretion; and/or (iii) differences in the regulatory actions of peripheral factors (mainly gonadal peptides, such as inhibins) that selectively impinge on FSH secretion. In any event, besides physiological interest, the above observations pose obvious pharmacological implications (e.g., design of protocols of selective activation of LH secretion), specially considering that the patterns of FSH responses to kisspeptin-10 appear to diverge between male (delayed but long-lived) and cyclic female (rapid but of shorter duration) rats, and depend on gonadal factors (our unpublished observations).

The mechanisms whereby kisspeptins stimulate gonadotropin secretion have been also the subject of intense investigation during the last 3 years. Of note, initial hormonal analyses of mice carrying inactivating mutations of the GPR54 gene already revealed that the basis for their HH

is not the abnormal migration of GnRH neurons from the olfactory placode during early stages of development (as it is the case in classical forms of Kallmann syndrome) [104]; a contention that has been recently confirmed by similar observations in KiSS-1 null mice [21]. Yet, it cannot be excluded that the constitutive lack of KiSS-1/GPR54 signaling throughout development might result in (subtle) modifications in dendritic arborization or synaptogenesis of GnRH neurons, although KiSS-1 knock-out mice show preserved LH responses to exogenous kisspeptin [21]. Likewise, analyses in mice and/or humans with genetic inactivation of GPR54 demonstrated that their state of hypogonadotropism is not due to a disturbed capacity to synthesize GnRH at the hypothalamus (*mouse*) or to respond to the decapeptide in terms of gonadotropin secretion at the pituitary (*mouse/human*) [104]. Collectively considered, those observations suggested that GPR54 (and its ligands) are rather involved in the dynamic control of the GnRH system, whose function appeared to be shut-down in conditions of defective GPR54 signaling [104]. Accordingly, it was anticipated that GnRH may operate as mediator for the stimulatory effects of kisspeptins on gonadotropin secretion. Indeed, this contention was fully confirmed by experiments of pharmacological antagonization of GnRH in the rat, mouse and monkey, where the ability of kisspeptin to elicit LH (and FSH) secretion was completely abrogated [33,60,70,71,106].

More direct evidence for the capacity of kisspeptins to stimulate GnRH secretion at the hypothalamus came from functional studies in the rat (*ex vivo*) and the sheep (*in vivo*); on the former, kisspeptin-10 was shown to elicit GnRH release by rat hypothalamic explants [10,11,73,123], while on the latter, intracerebral injection of kisspeptin induced the release of GnRH into the cerebrospinal fluid [63]. Altogether, these data strongly suggested that the primary site of action of kisspeptins in the control of the gonadotropic axis is located at hypothalamic GnRH neurons. Further support to this contention came from expression and functional studies, which showed that in the rat 77% of GnRH neurons co-express GPR54 mRNA, and that kisspeptin efficiently induced *c-fos* expression (as early marker of activation) in >85% of GnRH neurons [44]. In addition, the ability of kisspeptin to stimulate GnRH neurons was confirmed by electrophysiological recordings *in situ*, which showed that kisspeptin was capable to evoke long-lasting depolarization responses in >90% of this neuronal population in adult mouse hypothalamus [36]. In sum, compelling evidence demonstrates that kisspeptins are able to directly activate hypothalamic GnRH neurons, as major mechanism of action for their potent effects in terms of induction of gonadotropin release.

Further evidence for the physiological relevance of the hypothalamic kisspeptin/GPR54 system in the control of the gonadotropic axis was provided by expression analyses in models of gonadectomy. Thus, elimination of gonadal factors in male and female rats was initially shown to induce a significant rise in KiSS-1 mRNA levels in whole

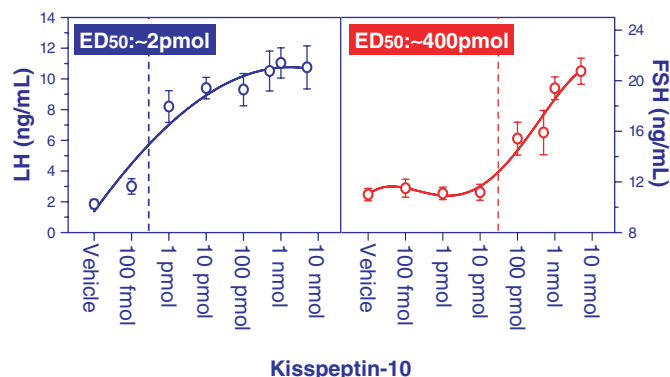


Fig. 3. Comparative analysis of the patterns of LH and FSH responses to intracerebral administration of increasing doses of kisspeptin-10 in the rat. Although secretion of both gonadotropins is maximally elicited by effective doses of kisspeptin-10, significant differences are noted in terms of median and minimal (threshold) effective doses, with LH secretion being approximately 200-fold more sensitive to kisspeptin stimulation than FSH. ED₅₀ values, as well as predicted minimal effective doses (*dotted lines*), are indicated for both gonadotropins. Composed from [70] and our unpublished data.

hypothalamic fragments, which was coincident with the expected increase in circulating gonadotropin levels [69]. Moreover, sex steroid replacement of gonadectomized rats (testosterone in males; estradiol in females) totally prevented both hormonal (LH and FSH) and gene expression (KiSS-1) responses [69]. These data were later refined by *in situ* hybridization analyses in rodents, which showed that sex steroids are able to repress KiSS-1 gene expression selectively at the ARC [110,111]. These observations pointed out that KiSS-1 neurons at this hypothalamic site are under the regulation of sex steroids and are likely to operate as an important relay for conveying the negative feedback actions of androgen and estrogen on gonadotropin secretion [114]. Of note, the ability of androgen to suppress KiSS-1 mRNA levels in mediobasal hypothalamus has been recently reported also in orchidectomized monkeys [107], while hypertrophy and increased KiSS-1 gene expression has been detected in the hypothalamic infundibular (arcuate) nucleus of postmenopausal women and ovariectomized monkeys [98]. These observations demonstrate the conserved role of KiSS-1 neurons at the hypothalamic infundibular/arcuate nucleus in conveying the negative feedback effects of sex steroids also in primates. However, this aspect of the function of KiSS-1 system (i.e., feedback control) has turned out to be more complex, and certainly more fascinating, than initially assumed, as it is now recognized that additional populations of KiSS-1 neurons within the hypothalamus are oppositely regulated by gonadal steroids and (likely) involved in mediating the positive feedback actions of estrogen on LH secretion at the pre-ovulatory period in the cyclic female (see Section 5.1).

4.3. The KiSS-1/GPR54 system and the control of puberty onset

Another facet of the physiology of kisspeptin/GPR54 that was first explored after the disclosure of its reproductive dimension was the putative role of this system in the pubertal activation of the HPG axis. In fact, the central role of GPR54 and its ligands in puberty onset was already evidenced by the reproductive phenotypes of humans and mice with null mutations of GPR54, which showed a state of sexual immaturity [30,104]. Detailed reviews on the prominent function of KiSS-1 system in the regulation of puberty in primates and rodents have been recently published [88,117]. For the purpose of the present work, we summarize below some of the most relevant facets of this aspect of kisspeptin function.

- In the rat, a significant increase in KiSS-1 (and GPR54) mRNA levels is detected at the hypothalamus coinciding with the onset of puberty, which was preceded by low expression during the prepubertal period [69]. Grossly similar observations (increased expression of KiSS-1 mRNA at the hypothalamus during the transition from the juvenile to the mid-pubertal stage) have been obtained in primates [106].

- Acute pharmacological tests demonstrated that kisspeptin-10 (as full agonist of GPR54) is able to elicit robust LH and FSH responses in pubertal male and female rats [69–71]. Likewise, acute administration of kisspeptin stimulated LH secretion in juvenile monkeys [106].
- In keeping with acute experiments, repeated (chronic) administration of kisspeptin to immature female rats induced the precocious activation of the gonadotropic axis [72]. Similarly, repetitive administration of kisspeptin-10 to monkeys at the end of the juvenile phase of sexual maturation induced a sustained train of GnRH discharges, reminiscent of that found at puberty [89].
- In the rat and mouse, there is significant increase in the sensitivity of GnRH/gonadotropin system to kisspeptin along pubertal maturation, as evidenced by (i) the dramatic enhancement in the proportion of GnRH neurons that are able to respond to kisspeptin *in vitro* [36]; and (ii) the significant lowering in threshold doses of kisspeptin required to elicit robust (maximal) LH responses *in vivo* [10,36]. This phenomenon has been related to an increase in the efficiency of GPR54 coupling to its intracellular effectors along puberty [36].
- In the female mouse, kisspeptin neurons selectively at the AVPV show a striking pattern of postnatal development, with virtually no discernible KiSS-1 neurons during the infantile period and a consistent increase in kisspeptin-IR from the prepubertal stage to puberty onset [17]. In addition, the number of appositions between kisspeptin fibers (projecting from the AVPV) and GnRH cell bodies significantly increase along the pubertal transition [17]. Intriguingly, male mice show a developmental pattern similar to that of females, with increased kisspeptin-IR along pubertal maturation only in the AVPV [17]. This observation challenges the view (based on RNA data from *in situ* hybridization studies) that KiSS-1 neurons at the AVPV are scarce and of limited functional relevance in the male rodent (see Section 5.1). Moreover, these data raise the question of what is the physiological role of KiSS-1 neurons from the ARC in the central activation of the HPG axis at puberty; a question that merits further investigation.

Altogether, the above data are illustrative of the paramount importance and high degree of sophistication of the function of the kisspeptin/GPR54 system in the regulation of puberty onset in mammals. Overall, the mechanism whereby this system participates in the onset of puberty may include, at least, four major components: (i) an increase in the endogenous kisspeptin tone, which—if sufficient—can drive the GnRH/gonadotropin axis to a state of full activation; (ii) an elevation in the sensitivity to the stimulatory effects of kisspeptin in terms of GnRH/LH responses; (iii) an enhancement of GPR54 signaling efficiency coupled to a less consistent increase in GPR54 expression; and (iv) an increase in the number of kisspeptin projections to GnRH neurons from specific hypothalamic areas (e.g. AVPV). The latter phenomenon appears

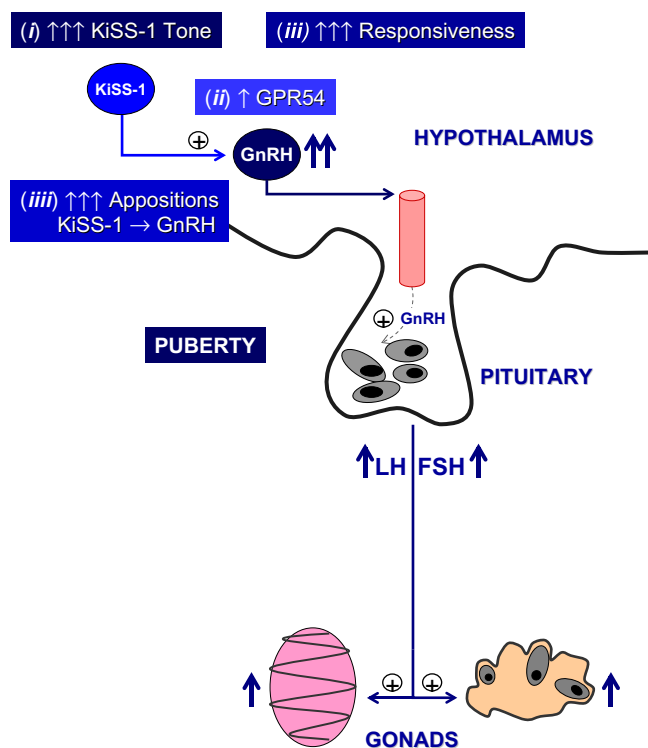


Fig. 4. Tentative model for the mechanisms potentially involved in the activation of GnRH neurons by kisspeptins and GPR54 at the time of puberty. These might include: (i) increase in endogenous kisspeptin tone at certain hypothalamic nuclei—likely AVPV; (ii) enhancement of the expression of GPR54, of lower magnitude than the ligand; (iii) increase in the sensitivity/responsiveness to the stimulatory effects of kisspeptin linked to augmentation of the efficiency of GPR54 coupling to its signaling systems; and (iv) increase in the number of appositions between KiSS-1 neurons (*projections*) and GnRH neurons. Overall, the enhancement in the activity of KiSS-1 system likely operates as a major driving signal for the full activation of the GnRH pulse generator (denoted by *arrows*), and subsequently gonadotropin secretion and gonadal function, at the time of puberty. For further details, see Section 4.3.

relevant for the precise timing of puberty, as GnRH/LH responsiveness to kisspeptin is present at earlier stages of postnatal development (e.g. neonatal/infantile period) [10,73]. The proposed quadri-partite model of action of kisspeptins and GPR54 in the regulation of puberty onset in mammals is depicted in Fig. 4.

5. The KiSS-1/GPR54 system and the neuroendocrine control of ovulation

One of the aspects of kisspeptin/GPR54 physiology that has drawn considerable attention recently is the specific role of this system in the regulation of female HPG axis in general, and in the neuroendocrine control of ovulation in particular. As indicated in Section 2, the female gonadotropic axis possesses some specific functional features that reflect a higher degree of sophistication of its regulatory mechanisms. These include the generation of the pre-ovulatory surge of gonadotropins, as hormonal trigger for ovulation. This is a complex neuroendocrine phenomenon,

highly sensitive to different environmental cues and endogenous factors, which involves a cascade of events, such as the pre-ovulatory rise in estradiol secretion by dominant follicles of the ovary followed by the increase in hypothalamic GnRH secretion (positive feedback) and GnRH self-priming at the pituitary [28,101]. Identification of the functional properties of the hypothalamic KiSS-1 system, as reviewed in previous sections, made it tempting to propose that kisspeptins and GPR54 might be involved in the generation of the pre-ovulatory surge of gonadotropins. However, little attention was initially paid to the characterization of the gonadotropin-releasing effects of kisspeptins in the adult cyclic female. Moreover, initial expression analyses, using whole hypothalamic fragments, failed to provide direct evidence for a putative role of KiSS-1 in the generation of the pre-ovulatory surge, as estrogen appeared to inhibit (negative feedback) rather than stimulate (positive feedback) the hypothalamic expression of KiSS-1 gene [72]. Further refinement of those original studies, by means of additional pharmacological and functional tests and *in situ* hybridization analyses, have now substantiated a fundamental role of kisspeptin/GPR54 in the reproductive female; the major characteristics of this key function will be reviewed in the following sections.

5.1. The hypothalamic KiSS-1 system and positive feedback of estradiol in the female

As stated above, the initial observation that estrogen represses the hypothalamic expression of KiSS-1 gene in female rats appeared to suggest a predominant role of this system in mediating the negative feedback of sex steroids [72]. Yet, although the importance of KiSS-1 neurons at the ARC in conveying the inhibitory effects of estrogen on gonadotropin secretion has been fully confirmed [110,111], detailed *in situ* hybridization analyses in rodents unveiled a higher level of complexity in the hormonal regulation of this hypothalamic system, as KiSS-1 neurons in the AVPV were shown to behave in a strictly opposite manner: expression of KiSS-1 mRNA at this site decreased after gonadectomy and markedly increased following estrogen replacement [110,111]. Considering that the AVPV had been previously involved in mediating the positive feedback effects of estrogen upon GnRH and LH surges, this was the first evidence to suggest that KiSS-1 neurons at this hypothalamic site might be involved in the generation of the pre-ovulatory gonadotropin surge, at least in rodents. In good agreement, the population of KiSS-1 neurons at the AVPV has been proven sexually dimorphic in the rat and mouse; it being much more abundant in the female (where positive feedback is detected) than in the male (devoid of positive feedback) [46,110].

Further evidence for the importance of the hypothalamic KiSS-1 system, and specifically of KiSS-1 neurons at the AVPV, in this phenomenon has been added recently in rodents. First, immunoneutralization of endogenous

metastin/kisspeptin was shown to block the pre-ovulatory LH surge and to disrupt estrous cyclicity in the rat [47]. Second, it was recently reported that KiSS-1 mRNA levels at the AVPV (but not at the ARC) increase dramatically during the evening of proestrus in the rat; i.e. coinciding with the pre-ovulatory surge of LH [112]. Likewise, standard estrogen and progesterone priming to ovariectomized female rats induced not only the expected surge of LH levels but also enhanced KiSS-1 expression at the AVPV [112]. In addition, most of KiSS-1 neurons at this hypothalamic site were activated (as indicated by the expression of *c-fos*) at proestrus and expressed estrogen receptor (ER)- α , which provides the basis for direct (positive) effects of estrogen on this neuronal population [112]. These observations in the rat, which have just been independently confirmed [1], together with recent immunohistochemical studies on the distribution and projections of KiSS-1 neurons in the forebrain of the mouse [17], have provided compelling evidence for a discernible neuronal network involved in mediating the positive feedback effects of estradiol of gonadotropin secretion, at least in rodents; a pathway that had remained elusive for decades. Indeed, recent functional studies using mice genetically engineered to lack functional ER α in neurons suggested that a population of, as yet undefined, neurons at the AVPV expressing ER α directly innervate GnRH neurons, and are likely indispensable for the positive feedback effects of estradiol and the generation of the LH surge [128]. On the basis of the above evidences, it is tempting to propose that such a population is composed of KiSS-1 neurons. Notwithstanding, it is worthy noting that the mechanisms for the generation of the preovulatory surge of gonadotropins are likely to differ between rodents and primates (see Section 5.3), and accordingly the actual roles of different populations of hypothalamic KiSS-1 neurons in such a phenomenon in higher primates (including the human) remains to be defined. Indeed, as is the case in the sheep [91], hypothalamic KiSS-1 neurons in primates appear mostly located at the infundibular (arcuate) nucleus—not at the AVPV, but their potential contribution to the rise of gonadotropins at the preovulatory period has not been assessed so far.

5.2. Responsiveness to kisspeptin in the female: effects of cycle, pregnancy and lactation

Besides fluctuations in KiSS-1 expression at certain hypothalamic nuclei, an additional source for cyclic changes in gonadotropin secretion in the female might stem from differences in the responsiveness to endogenous kisspeptin. To explore such a possibility, we have recently evaluated LH secretory responses to intracerebral injection of kisspeptin-10 in adult female rats, at different phases of the ovarian cycle [97]. These analyses demonstrated that, although robust LH responses to kisspeptin were observed across the cycle, maximal LH secretion is selectively induced by kisspeptin during the transition of proestrus

to estrus. Such a window of maximal effectiveness for the LH-releasing effects of kisspeptin is likely defined by changes in the sex steroid milieu at certain stages of the cycle. This hypothesis is supported by data from pharmacological tests in ovariectomized rats that demonstrated that combined replacement with estradiol and progesterone was able to induce maximal LH responses to kisspeptin, similar to those observed at the proestrus-to-estrus transition [97]. Overall, it is tempting to propose that, in addition to activate KiSS-1 neurons at the AVPV, the rise in estradiol levels during proestrus, in the presence of activated progesterone receptors, evokes a state of hyper-responsiveness to kisspeptin that is likely to contribute to the generation of the pre-ovulatory surge of gonadotropins. A tentative model for the multi-faceted interplay between sex steroids and the KiSS-1 system in the generation of the pre-ovulatory peak of LH is shown in Fig. 5. In addition, results from functional tests in female rats further

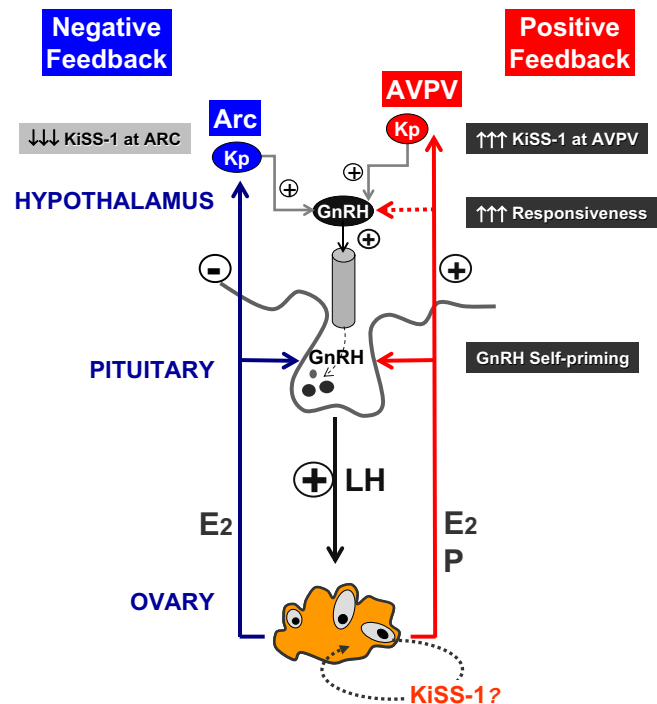


Fig. 5. Tentative model for the role of KiSS-1/GPR54 system in the generation of the pre-ovulatory surge of LH and the neuroendocrine control of ovulation. Estrogen (E2), in the presence of activated receptors for progesterone (P), is mandatory for induction of the pre-ovulatory surge of LH (*positive feedback*); a phenomenon that involves: (i) activation of kisspeptin (Kp) neurons at the anteroventral periventricular nucleus (AVPV) at the time of the surge; and (ii) a state of enhanced responsiveness to kisspeptin, likely at the level of GnRH neurons, during the peri-ovulatory period. In addition, ovarian steroids are essential for the modulation of (iii) pituitary responsiveness to GnRH and GnRH self-priming. For comparison, the proposed role of KiSS-1 neurons at the arcuate nucleus (ARC) in mediating the *negative feedback* effect of E2 on gonadotropin secretion—outside the preovulatory period—is also presented. In addition, recent data evidenced the expression of KiSS-1 and GPR54 at the ovary (rat and human), which opens up the possibility of additional, direct effects of kisspeptins in the local control of ovulation. For further details, see Section 5.4.

stress the need for a precise temporal regulation of kisspeptin inputs on GnRH neurons as absolutely mandatory for the generation of the surge in the evening of proestrus and its termination at early estrus, in keeping with data from expression analyses [112].

Admittedly, the changes in the magnitude of LH responses to kisspeptin along the estrous cycle and after manipulation of ovarian sex steroid levels summarized above might derive from fluctuations in the sensitivity of GnRH neurons to kisspeptin and/or changes in pituitary sensitivity to GnRH. On the latter, an increase in the pituitary sensitivity due to GnRH self-priming has been reported at proestrus [15]; a phenomenon which critically depends on the activation of estrogen and progesterone receptors. Yet, recent data obtained at our laboratory strongly suggest that, in addition, sex steroid inputs might modulate the responsiveness of GnRH neurons to kisspeptin stimulation [our unpublished results].

In addition to ovarian cycle, the female gonadotropic axis undergoes significant functional fluctuations during pregnancy and lactation when, among others, clear-cut changes in basal secretion of gonadotropins are detected in pregnant and lactating dams, with a marked suppression of the axis especially at lactation [113]. Studies on the hypothalamic expression and function of the KiSS-1/GPR54 system have been recently initiated in order to define the potential contribution of kisspeptin signaling to the regulation of gonadotropin secretion at those states. Regarding pregnancy, a dramatic increase (>7000-fold) in circulating levels of metastin has been reported in pregnant women [41], despite the suppression of circulating gonadotropin levels detected during gestation in the human [95]; an observation which is suggestive of a state desensitization of gonadotropin responses after exposure to persistently elevated levels of kisspeptin. Contrary to that hypothesis, in pregnant rats, at mid- and late-gestation, net LH responses to effective doses of kisspeptin-10 were not only conserved, but actually enhanced. Moreover, the sensitivity to low doses of kisspeptin-10 was preserved (in terms of LH secretion) at mid-gestation, with effective doses as low as 0.1 pmol/i.c.v. [97]. In good agreement, the expression levels of GPR54 mRNA at hypothalamus did not significantly decrease in pregnant rats, while hypothalamic expression of KiSS-1 gene actually increased along pregnancy; a phenomenon whose functional relevance is yet to be defined. Overall, those data suggest that, at least in the rat, there is no significant desensitization to the stimulatory effects of kisspeptin on gonadotropin secretion during gestation [97]. Of note, however, the actual circulating levels of metastin/kisspeptin during pregnancy in the rat have not been reported so far. Thus, it remains to be confirmed whether, as is the case in humans, plasma concentrations of KiSS-1 peptides raise during rat gestation.

Finally, the potential contribution of changes in kisspeptin/GPR54 signaling to the marked suppression of the HPG axis during lactation has been explored recently. Our studies demonstrated that intracerebral injection of

high doses of kisspeptin are able to elicit LH secretory responses in lactating dams [97]; a phenomenon that has been confirmed recently [129]. Yet, the net magnitude of these responses was significantly attenuated in (intact) lactating dams. Moreover, the sensitivity to kisspeptin in terms of gonadotropin secretion was also significantly decreased during lactation, as doses of 10 pmol kisspeptin-10 (i.c.v.) were unable to elicit LH secretion in lactating rats whereas doses of 0.1 pmol were effective in cyclic (and pregnant) rats [97]. In addition, recent data from lactating-ovariectomized rats suggest that KiSS-1 mRNA levels and kisspeptin immunoreactivity are suppressed at the ARC during lactation [129]. Altogether, the above findings strongly suggest that changes in kisspeptin signaling might be mechanistically relevant for the suppression of the gonadotropic axis during lactation, and likely involve two major components: (i) a significant suppression in the sensitivity of the gonadotropic system to kisspeptin stimulation, with an elevation of the threshold effective doses of at least two orders of magnitude; and (ii) a decrease of the endogenous kisspeptin tone at discrete hypothalamic areas, such as the ARC.

5.3. Sexual differentiation of hypothalamic KiSS-1 system: role in the pre-ovulatory surge

Cyclic function of the female gonadotropic axis in adulthood, with positive feedback effects of estradiol and the generation of the pre-ovulatory LH surge, critically depends, at least in rodents and sheep, on early developmental events driven by gonadal steroids during critical periods of sexual differentiation; a period which in the rat spans during the neonatal age [119]. Thus, exposure to high levels of testosterone (and its metabolite estradiol, generated by local aromatization) at early postnatal life results in the ‘masculinization’ of the neuronal circuitry in the developing hypothalamus [66]. Conversely, low estrogen exposure seems mandatory for ‘feminization’ of the developing brain. Of note, however, the mechanisms for such a sexual differentiation of gonadotropin control might be partially different between rodents and primates, as LH surges can be induced in male monkeys—not subjected to hormonal manipulation at early stages of development, if provided with an appropriate ovarian (estrogen) signal as adults [76].

In the above context, analysis of sexual differentiation of the hypothalamic KiSS-1 system has been recently initiated in the rat [46]. Results from those studies demonstrate that development of KiSS-1 neurons at the AVPV, but apparently not at the ARC, is sensitive to the organizing effects of sex steroids during the neonatal period of sexual maturation of the brain. Thus, neonatal androgenization of female rats resulted in the complete ‘masculinization’ of the pattern of expression of KiSS-1 at the AVPV in adulthood since, contrary to cyclic females, (i) neonatally androgenized female rats displayed negligible expression of KiSS-1 mRNA at this hypothalamic site (as it is the case in adult males); and (ii) exposure to estrogen as adults failed to increase KiSS-1

mRNA expression at the AVPV of neonatally androgenized females [46]. Considering that neonatal androgenization of the female rat results also in the prevention of the positive feedback effects of estradiol and lack of LH surges, the above data add further strength to the contention that KiSS-1 neurons at the AVPV, which are sexually dimorphic and developmentally regulated, subserve the cellular mechanisms controlling the generation of the pre-ovulatory surge of GnRH/LH, selectively in the female. From a more general perspective, those observations emphasize the sensitivity of the hypothalamic KiSS-1 system to the organizing actions of endogenous (and eventually exogenous) sex steroids. This is in good agreement with results from our group showing that neonatal exposures to synthetic estrogens, known to disturb proper activation and function of the gonadotropic axis [119], persistently suppressed the hypothalamic expression of KiSS-1 gene at the expected time of puberty and adulthood in the rat [Tena-Sempere, our unpublished observations].

5.4. Ovarian expression of the KiSS-1 system: role in the local control of ovulation?

Further complexity of the potential actions of the KiSS-1/GPR54 system in the control of ovulation has been recently suggested by the demonstration of the expression and hormonal regulation of KiSS-1 gene at the ovary. In fact, the ovarian expression of KiSS-1 and GPR54 mRNAs had been preliminarily reported previously [121]. We have now deepened those initial observations, providing compelling evidence for the presence of the elements of the KiSS-1 system in the rat ovary, with detectable mRNA levels of KiSS-1 and GPR54 across the estrous cycle, and clear-cut kisspeptin-IR in the theca layer of growing follicles, corpora lutea and interstitial gland; compartments where modest GPR54 immunoreactivity was also observed [9]. Interestingly, KiSS-1 (but not GPR54) mRNA expression in the ovary has been shown to change in a cyclic-dependent manner, with a marked increase in the afternoon of proestrus, i.e., preceding ovulation. Such a rise in the ovarian levels of KiSS-1 mRNA is likely driven by the pre-ovulatory surge of gonadotropins as (i) it was prevented by blockade of this surge by means of pre-treatment with an antagonist of GnRH; and (ii) it was induced by administration of human CG, as super-agonist of LH, to female rats treated with a GnRH-antagonist [9]. In keeping with those observations in the adult ovary, immature rat ovaries showed low to negligible levels of KiSS-1 mRNA, but expression of KiSS-1 was significantly enhanced by a standard protocol of gonadotropin priming [9]. Although the physiological relevance of the presence of kisspeptins and GPR54 in the ovary remains to be elucidated, the ability of the LH surge to timely induce the ovarian expression of KiSS-1 gene at the pre-ovulatory period suggests that locally produced kisspeptins might be involved in the control of some aspects of ovulation. In addition, expression of KiSS-1 mRNA and peptides, in a regionalized and cyclic-

dependent manner, has been very recently reported in the rat oviduct; an observation that raises the intriguing possibility of a putative role of KiSS-1 in the prevention of ectopic (tubal) implantation in rodents [31]. Obviously, the above findings do not invalidate the consensus that the major site of action of kisspeptins/GPR54 in the neuroendocrine regulation of ovulation is located at the hypothalamus, but adds another facet to the complex mode of action of kisspeptins in the control of reproduction; a facet that might have been initially overlooked due to the prominent roles of this system at central (hypothalamic) levels (see Fig. 5).

6. The KiSS-1/GPR54 system and the metabolic control of reproduction

The pivotal role of kisspeptins and GPR54 in the control of GnRH neurons, and hence of the HPG axis, as reviewed in previous sections, prompted the analysis of the potential function of this system in mediating the actions of well-known regulators of the reproductive axis, other than sex steroids. Reproduction is highly sensitive to the state of energy reserves and the metabolic status of the organism; sufficient energy stores and proper metabolic status being mandatory for fertility. The neuroendocrine basis for such a connection has been (partially) deciphered in recent years, when the roles of different 'metabolic' hormones in the integrated control of energy balance and reproduction have been characterized [27]. Among those, a fundamental role of leptin, a hormone secreted by the adipose tissue that signals the state of energy reserves to the brain centers governing reproduction, has emerged; leptin being now recognized as an essential permissive signal for puberty onset and maintenance of reproductive capacity in adulthood [8,27]. However, although the effects of leptin and other hormones, arising from the adipose tissue—e.g., adiponectin, the pancreas—e.g., insulin— and the gastrointestinal tract—polypeptide YY3-36 and ghrelin—on the HPG axis have been well characterized over the past years [27], the central circuits transmitting such energy/metabolic information onto the centers governing reproduction remain (partially) undefined.

6.1. Energy balance and metabolic status influence the hypothalamic KiSS-1 system

Demonstration of a potential role of the hypothalamic KiSS-1 system in conveying information regarding metabolic and energy status was originally based in two premises: (i) conditions of altered energy homeostasis or metabolism known to have an impact on the gonadotropic axis should influence also the expression of KiSS-1 at the hypothalamus; and (ii) exogenous administration of kisspeptins in states of metabolic impairment should ameliorate or totally rescue defective gonadotropic function in those conditions. These assumptions have been now supported by several lines of evidence.

- Protocols of short-term fasting, which induced a reduction in basal gonadotropin levels, evoked also a significant decrease in the expression of KiSS-1 mRNA at the hypothalamus of pubertal males and female rats [11]. These findings have been recently replicated in adult male mice [56].
- Acute intracerebral administration of kisspeptin-10 to fasted rats was able to reverse the state of hypogonadotropism induced by food-deprivation and evoked LH responses that were augmented over those detected in control animals fed *ad libitum*. The mechanism for such exaggerated responses might be related to an increase in the hypothalamic expression of GPR54 observed in fasted rats, which is likely due to a primary decrease in endogenous kisspeptin tone [11].
- Repeated (chronic) administration of kisspeptin in a model of under-nutrition of immature female rats (30% restriction of calorie intake, which prevented puberty onset) was sufficient to restore vaginal opening (as external index of puberty) in a significant number of animals (60%), and induced robust gonadotropin and estrogen responses, despite 30% reduction in body weight and in absence of other metabolic interventions [11].
- In a rat model of metabolic disturbance linked to hypogonadotropism, such as experimental diabetes induced by injection of streptozotocin (STZ), basal expression of KiSS-1 gene at the hypothalamus was significantly decreased [12].
- In diabetic STZ-rats, the expected diminished response to gonadectomy in terms of elevation of serum LH levels (as index of hypogonadotropism) was associated to a blunted rise in hypothalamic KiSS-1 mRNA levels post-gonadectomy [12].
- Acute intracerebral administration of kisspeptin-10 to diabetic rats was capable to reverse the state of hypogonadotropism induced by uncontrolled diabetes and induced LH and testosterone responses that were, at the very least, similar to those observed in control animals [12].
- Chronic administration of kisspeptin to STZ-injected rats was sufficient to ameliorate the state of hypogonadotropic hypogonadism (enhancement of prostate and testis weights; normalization of LH and testosterone levels) induced by uncontrolled diabetes, in spite of the absence of any metabolic intervention [12].

Altogether, the above findings suggest that the metabolic status of the organism is a major regulatory factor of the hypothalamic KiSS-1 system. Accordingly, it is tempting to hypothesize that KiSS-1 neurons in the forebrain may operate as sensor for altered energy balance and metabolic impairment; conditions that cause a decrease in hypothalamic kisspeptin tone. Thus, kisspeptins (and GPR54) appear to act as key molecular conduits for relaying information concerning energy balance onto the centers (likely GnRH neurons) governing the gonado-

tropic axis, thereby contributing to the coupling of the reproductive capacity to the state of body energy stores and metabolic status.

6.2. *Leptin is a major metabolic regulator of the hypothalamic KiSS-1 system*

Although the hypothalamic KiSS-1 system was proven sensitive to the nutritional and metabolic state of the organism, the question arising from the above observations is what are the neuroendocrine signals and circuitries responsible for conveying such metabolic information onto KiSS-1 neurons in the forebrain. Although this key aspect of kisspeptin physiology remains to be fully solved, some recent evidence suggests that the adipose hormone, leptin, plays a dominant role in this phenomenon. Indeed, experimental work gathered in the last 10 years had well characterized the prominent function of leptin in signaling the magnitude of energy (fat) reserves to reproductive centers, thus serving an indispensable role for proper puberty onset and maintenance of reproductive capacity in adulthood [8,27]. The possibility that leptin might be afferent to KiSS-1 neurons at the hypothalamus was indirectly suggested by the observation that conditions of hypo-gonadotropism due to absence of leptin actions could be rescued by exogenous administration of kisspeptin; the first evidence to indicate that the KiSS-1/GPR54 system is distal to (or eventually independent of) leptin in the control of the gonadotropic axis [72]. More recently, two independent pieces of evidence have been presented, which strongly suggest that leptin is a putative regulator of the hypothalamic KiSS-1 system:

- In leptin deficient gonadectomized ob/ob mice, the expression of KiSS-1 gene at the ARC was significantly decreased [108]; a phenomenon that was partially reversed by acute intracerebral administration of leptin.
- In the model of uncontrolled diabetes (induced by STZ injection), linked to hypo-leptinemia, defective KiSS-1 gene expression at the hypothalamus could be normalized by chronic intracerebral infusion of leptin (but not insulin). This response was associated to a significant elevation of circulating LH and testosterone, which reached levels similar to those of control animals [12]. These observations are in line with very recently published evidence showing that, in the murine hypothalamic cell line N6, expression of KiSS-1 gene is up-regulated by leptin, but not insulin [56].

Considering, in addition, that it has been recently reported that a substantial proportion of KiSS-1 neurons at the ARC express the leptin receptor gene [108], it is tempting to propose that peripheral leptin levels (as signal for the magnitude of body energy stores) may modulate the functioning of the reproductive axis (and specifically, of GnRH neurons) via regulation of the hypothalamic KiSS-1 system, likely at the ARC. The potential regulation,

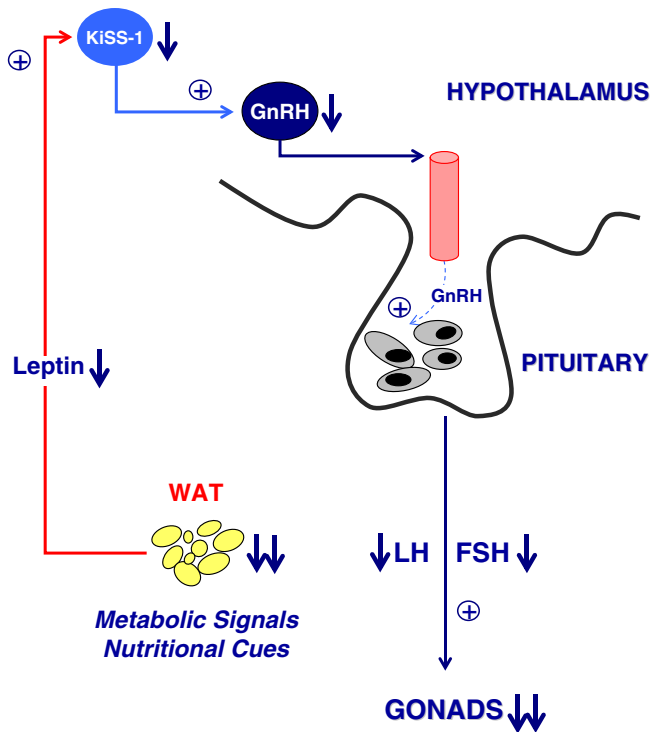


Fig. 6. Tentative model for the role of KiSS-1 neurons as central integrators of energy balance and reproduction. Expression and function of KiSS-1 system at the hypothalamus appears sensitive to the state of energy reserves and metabolic factors. Thus, conditions of disturbed energy balance (e.g. undernutrition) or impaired metabolic state (e.g. experimental diabetes)—denoted by *downside arrows*—induce a decrease in KiSS-1 expression at the hypothalamus, which in turn causes a state of hypogonadotropic hypogonadism. Leptin, a hormone produced by the white adipose tissue (WAT) in proportion of fat mass, plays a crucial role in relaying information of the metabolic state of the organism onto KiSS-1 neurons and, hence, the reproductive axis. Taken from [116], with modifications.

if any, of KiSS-1 neurons at the AVPV by leptin remains to be analyzed. In any event, such leptin–kisspeptin connection would help to solve the apparent conundrum that, while it being absolutely essential for the control of the gonadotropic axis, GnRH neurons in the forebrain do not apparently express leptin receptors in physiological conditions [27]. A tentative model for the role of KiSS-1 neurons as central integrators of energy balance and reproduction, and the essential function of leptin in such neuroendocrine circuitry is depicted in Fig. 6.

7. Comparative endocrinology of the KiSS-1/GPR54 system

Despite the contention that kisspeptins and GPR54 are posed with essential functions in the control of reproductive axis, which appear to be conserved between humans and rodents [104], it is noticeable that most of the experimental work conducted so far in this area has been restricted to mammalian species (mainly, rats, mice and primates), where genetic studies, expression analyses and pharmacological tests have been conducted (*see previous*

sections). In contrast, very limited information has been reported to date on the structural and functional characteristics of kisspeptins and GPR54 in non-mammals. Taking into account the paradigmatic example of GnRH and its receptor, whose functional diversity—even in mammals—was exposed by the identification of isoforms of the classical ligand (GnRH-I) and receptor in different species (e.g., GnRH type-II or chicken GnRH and GnRH-III or salmon GnRH), it is tempting to propose that detailed comparative analyses of KiSS-1 and GPR54 in a diversity of mammalian and non-mammalian species would be utmost helpful, not only to define the phylogeny of this system, but also to refine its functional characterization in terms of control of fertility and, eventually, other biological actions.

7.1. Structural analyses

The cDNA sequences of KiSS-1 from a number of mammalian species have been cloned to date. These include the human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), macaque (*Macaca mulatta*), bull (*Bos taurus*), sheep (*Ovis aries*), rat (*Rattus norvegicus*) and mouse (*Mus musculus*) (GenBank Accession Nos. NM_002256, XM_514123, AY_823262, XM_867473, DQ_059506, NM_181692, and NM_178260, respectively). Alignment of the predicted amino acid sequences demonstrates a high degree of homology among primate species (>85%), whereas sequence identity between the human, bovine, ovine and rodent kisspeptins ranges between 45% and 50% [96]. To our knowledge, no cDNA or protein sequences of KiSS-1/kisspeptins from non-mammalian species have been annotated so far in gene databases. Concerning GPR54, the cDNA sequences from different mammalian and non-mammalian species have been reported to date. These include (among mammals) the human, macaque, boar (*Sus scrofa*), rat and mouse (GenBank Accession Nos. NM_032551, AY_833261, DQ_459345/46, NM_023992, and NM_053244, respectively), as well as several non-mammalian GPR54s, such as those from the zebra fish (*Danio rerio*; GenBank Accession No. XM_685300), tilapia (*Oreochromis niloticus*; AB_162143), grey mullet (*Mugil cephalus*) [75], cobia (*Rachycentron canadum*) [65], Atlantic croaker (*Micropogonias undulatus*; ABC75101.1), bullfrog (*Rana catesbeiana*) [20], and purple sea urchin (*Strongylocentrotus purpuratus*; XM_001188545). Alignment of the predicted amino acid sequences of GPR54 from mammalian and non-mammalian species reveals that the percentage of homology between primates and rodent sequences is much higher for GPR54 (>80%) than for KiSS-1 peptide. Moreover, sequence identity between human, fish and frog GPR54 is >45%, while the homology between the human and urchin receptors is >20%. These data suggest a higher degree of sequence conservation for the receptor than for ligand in the KiSS-1/GPR54 system during evolution [96].

7.2. Expression and functional studies

Whereas our knowledge on structural aspects of KiSS-1 and GPR54 sequences in different species has considerably enlarged recently, functional analyses on the expression and function of this system remains mostly restricted to primates and rodents. An exception to this ‘trend’ comes from studies on the expression of KiSS-1 gene and effects of kisspeptin on GnRH and LH secretion in the sheep. While the latter have been mostly confirmatory of the ability of kisspeptins to potently elicit GnRH and gonadotropin secretion in other mammals [3,63], the former have pinpointed interesting species differences in terms of the anatomical distribution and functional organization of the elements of the KiSS-1 system at the hypothalamus between otherwise phylogenetically proximal species. Thus, while in female rodents a prominent population of KiSS-1 neurons, with a pivotal role in the generation of the pre-ovulatory LH surge, has been identified at the AVPV (see Section 5.1), such a population is not detectable in the sheep [91], where the neuronal network subserving the generation of positive feedback of estrogen appears to include a population of KiSS-1 neurons located in the caudal region of the ARC [26,109]. Other interesting species difference is related to the presence of a population of kisspeptin-expressing neurons selectively in the preoptic area of the sheep; neurons that do express estrogen receptors (ER- α) [29]. Interestingly, the presence of neurons co-expressing GnRH and kisspeptin has been recently reported in this hypothalamic area in the sheep [91]. If fully confirmed, this finding would predict additional mechanisms of action of kisspeptin in the control of gonadotropin secretion, which might involve direct pituitary effects after its co-release with GnRH into the hypophyseal portal blood system (see also Section 8).

Concerning non-mammalian species, despite the paucity of functional data, some findings reported over the last years strongly suggest the conserved role of GPR54 in the control of reproduction along evolution. First, expression of GPR54 gene has been demonstrated in GnRH neurons of cichlid fish, with higher percentage of expression in mature GnRH neurons (45–60%) than in immature neurons (5%) [85]. More recently, expression of GPR54 gene in the brain of grey mullet has been shown to be significantly augmented at the early stages of puberty [75]. Likewise, a concomitant increase in the brain levels of GPR54 and GnRH mRNAs has been reported during early puberty in the teleost fish cobia [65]. Altogether, the above similarities in the patterns of expression of GPR54 (e.g., presence of GPR54 in GnRH neurons; increased expression preceding puberty onset) point out that GPR54 and its ligand(s) are likely to conduct essential roles in the regulation of the reproductive axis in phylogenetically divergent species, such as fish and mammals. Another interesting aspect arising from comparative analyses is that the elements of the KiSS-1/ GPR54 system are present in the ovary not only in mammals (rat [9]; human [our unpub-

lished data]) but also in the fish grey mullet, where expression of GPR54 mRNA at the ovary increased at the intermediate stage of puberty [75]. The functional significance of the ovarian expression of GPR54, and its apparent conservation along evolution, awaits to be elucidated (see also Section 5.4).

8. Current challenges and future directions in kisspeptin physiology

Despite their reproductive dimension remained unnoticed prior to late 2003, the advancement of our knowledge on the ‘reproductive physiology’ of kisspeptins and GPR54 has been astonishingly rapid. Nonetheless, important aspects of their role in the control of key facets of reproductive function remain partially unsolved and are yet to be fully elucidated. We provide in this section a tentative list of current challenges in kisspeptin physiology that, without being exhaustive, might help to delineate future research lines in this field.

- *Physiology of KiSS-1 neurons in the forebrain.* While the location of KiSS-1 neurons in the forebrain of rodents (rat and mouse) and sheep has been well characterized recently, the afferents to and the projections of the different neuronal populations are yet to be defined. In this context, the use of pseudorabies virus tracing strategies coupled to transgenic approaches, allowing selective replication and retrograde transfer in GnRH or KiSS-1 neurons, would be extremely instrumental to define the actual neuronal networks involving kisspeptins controlling the reproductive axis. Likewise, there is a conspicuous lack of knowledge on the neuropeptide systems responsible for the control of KiSS-1 gene expression and kisspeptin release at the hypothalamus. Deciphering of both KiSS-1 networks and their regulation is mandatory to fully characterize the mode of action of kisspeptins and GPR54 in the control of the gonadotropic axis.
- *Other sites of action of kisspeptins in the HPG axis.* The contention that kisspeptins act primarily at the hypothalamus to conduct their regulatory actions of the gonadotropic axis is widely accepted. Yet, the possibility of additional effects of KiSS-1 peptides at other sites of the HPG axis remains contentious. For instance, prominent expression of GPR54 gene was initially demonstrated in human pituitary; yet, contradictory results on the ability of kisspeptins to modulate gonadotropin secretion directly at the pituitary level have been presented, with reports of either no effects or moderate stimulatory actions of kisspeptin upon LH and FSH release *in vitro* [60,70,71,123]. While some of these discrepancies might stem from methodological differences, an interesting possibility is that the ability of kisspeptins to directly elicit pituitary gonadotropin secretion may depend on the developmental stage tested, with modest, but significant, responses being detected at the pubertal

period; an option that is supported by our recent data in dispersed pituitary cell cultures from male and female rats at puberty [35]. In addition, the possibility of direct actions of kisspeptins in the gonads has been discussed (as referred to the ovary) on Section 5.4 of this review. Of note, expression of GPR54 and KiSS-1 genes has been detected in the testis [77] [Tena-Sempere, *in preparation*], and indirect evidence for direct effects of kisspeptins on the testis has been presented recently [92].

- *Mechanisms for the effects of estradiol and progesterone on KiSS-1 system.* As reviewed in previous sections, the ability of estrogen to differentially regulate the expression of KiSS-1 gene at the hypothalamus (stimulation at the AVPV; inhibition at the ARC) is now well characterized as major contributing factor for its positive and negative feedback effects on gonadotropin secretion. However, the molecular basis for such a dual (opposite) mode of action of estrogen remains unknown. In this sense, there is a conspicuous lack of studies on the functional characterization of the promoter of KiSS-1 gene; to our knowledge, only one paper has been published to date on this topic, in the context of the anti-metastatic actions of KiSS-1 [64]. In addition, whereas the role of progesterone in the expression of the pre-ovulatory LH surge is well established, the effects of progesterone on the regulation of KiSS-1 gene expression has not been studied in rodents, although some initial evidence for its ability to modulate KiSS-1 mRNA levels at the hypothalamus has been recently reported in the sheep [109]. Finally, although KiSS-1 neurons appear to be essential in the generation of the LH surge, it is yet to be defined whether and how additional signals (e.g., those entraining the surge to endogenous circadian rhythms) co-operate with kisspeptins in such a function remains to be elucidated.
- *Metabolic regulation of KiSS-1 system.* As indicated in Section 6, recent experimental data indicate that the metabolic state of the organism influences reproductive function via modulation of hypothalamic KiSS-1 system; a phenomenon which is mediated by the adipose hormone, leptin. However, it remains to be characterized whether such leptin actions are conducted only at KiSS-1 neurons located at the ARC (see Section 6.2), or involve also the population of kisspeptin neurons at the AVPV, which are abundant in the female rodent; females being far more sensitive than males to the metabolic control of fertility. Similarly, it is still an open issue whether the modulatory actions of leptin onto GnRH neurons are solely mediated via kisspeptin signaling, or whether additional signals, involved in the joint control of energy homeostasis and reproduction, cooperate with leptin in the metabolic regulation of KiSS-1 neurons. On the latter, leptin is thought to play a permissive (rather than trigger) role for puberty onset and reproductive capacity, which makes it tempting to hypothesize the need for a coordinated action of leptin with other central and/or peripheral regulators in the metabolic control of the gonadotropic axis; regulators

whose nature remains to be elucidated. Of note, recent studies suggest a potential role of neuropeptide Y (NPY) in the central control of hypothalamic KiSS-1 expression, since (i) KiSS-1 mRNA levels are persistently decreased in the hypothalamus of NPY knockout mice; and (ii) NPY increases KiSS-1 mRNA levels in the murine hypothalamic cell line, N6 [56]; yet, the physiological relevance of NPY in the metabolic regulation of the hypothalamic KiSS-1 system remains to be elucidated. Likewise, the molecular mechanisms whereby leptin, and eventually other metabolic signals, modulate the expression of KiSS-1 at the hypothalamus (allowing the effective coupling of energy stores and reproductive function) await to be characterized.

- *KiSS-1 system, photoperiodic cues and seasonal breeding.* In keeping with its central role in the control of the gonadotropic axis, it has been recently proposed that the hypothalamic KiSS-1 system participates in the photoperiodic control of reproduction in seasonal breeders, such as hamsters and sheep [34,93,94,109]. While this possibility is extremely appealing, some facets of such a function remain to be fully solved. For instance, the ability of melatonin to actually regulate KiSS-1 expression has been suggested based on indirect evidence but remains to be proved. Likewise, the functional anatomy of KiSS-1 neurons in the forebrain responsible for the seasonal control of reproduction is yet to be fully elucidated, as some discrepancies on the putative roles of ARC and AVPV KiSS-1 neurons have been reported in hamsters [34,94].
- *Comparative endocrinology of KiSS-1 system.* As indicated in Section 7, there is a significant paucity in functional data on the potential 'reproductive' roles of kisspeptins and GPR54 in non-mammalian species. This might be due to the lack of cloning of KiSS-1 gene in species other than mammals. Of note, however, the ability of rodent kisspeptin-10, albeit with higher EC50, to activate bullfrog GPR54 has been recently reported [20]. This finding poses two interesting implications: (i) it is possible to conduct heterologous hormonal tests (e.g., with rodent kisspeptin-10 in fish and amphibians) to identify potential roles of this system in the control of the reproductive axis in non-mammals; and (ii) the fact that the sensitivity of bullfrog GPR54 to rodent kisspeptin-10 is ~10-fold lower than that of the rat receptor is suggestive of the existence of a specific form of kisspeptin in the bullfrog, which is yet to be isolated. Overall, it is reasonable to anticipate that, as it was the case with the GnRH system, detailed comparative studies on the structure and function of KiSS-1/ kisspeptins and GPR54 in mammals and non-mammals species would help to complete the characterization of the reproductive (and eventually, non-reproductive) roles and modes of action of this ligand–receptor system in different species.
- *Pharmacological implications.* The extraordinary potency of kisspeptins in terms of activation of the HPG axis makes them (and GPR54) suitable targets

for potential therapeutic intervention in a wide range on pathological conditions, such as altered puberty, infertility and hormone-dependent cancer. In this context, generation of kisspeptin analogs, with agonistic or antagonistic activities and improved pharmacological profiles (e.g., concerning routes of administration or half-life), poses obvious interest. Indeed, this field has drawn quite some attention recently, and a number of kisspeptin analogs with agonistic activity at the GPR54 have been reported during the last year [74,81,124,125]. To our knowledge, however, (i) the ability of such compounds to stimulate the gonadotropic axis has not been so far tested *in vivo*; and (ii) no antagonists of GPR54 have been reported to date. In addition, in terms of pharmacological application, another important aspect to be elucidated is whether continuous administration of kisspeptins, or analogs, might induce desensitization to their effects in terms of gonadotropin secretion. In this sense, data reported recently strongly suggest that, as is the case for GnRH, the appearance of desensitization may depend on the pattern of kisspeptin stimulation. Thus, continuous infusion of kisspeptin-10 desensitized GnRH release in juvenile and adult rhesus monkeys, and induced a significant decrease in LH levels despite persistent exposure to kisspeptin [92,103]. Of note, such a suppression of the HPG axis disappeared shortly after cessation of kisspeptin infusion [103]. In contrast, a protocol of repeated administration of 4 boluses of kisspeptin-10 to male rats elicited a sustained pattern of LH pulses, without decrement in terms of peak amplitude, duration and secretory mass, and preserved LH responses in terminal GnRH tests [126]. Likewise, repetitive activation of GPR54 in juvenile monkeys (by means of brief kisspeptin boluses every hour for 48-h) evoked a persistent train of GnRH/LH discharges [89]. Taken together, these observations suggest that, depending on the pattern of GPR54 activation, the gonadotropic axis can be either maximally activated or reversibly suppressed by different patterns of kisspeptin administration; a phenomenon of as yet undefined pharmacological applications.

- **Clinical relevance of mutations in GPR54 and KiSS-1 genes.** Although very rare, data in the literature demonstrate that a sub-set of idiopathic forms of normosmic HH can be due to deletions and inactivating mutations in the GPR54 gene (see Fig. 2); yet, their frequency is lower than that of mutations in the GnRH receptor gene [14] and its epidemiological impact very limited. In contrast, not a single case of genetic inactivation of the KiSS-1 gene (as causative for HH) has been reported to date. Likewise, it remains to be determined whether mutations or polymorphic variations in the GPR54 and/or KiSS-1 genes might be linked to some forms of precocious puberty of central origin. On the basis of its physiological role, it is reasonable to predict that constitutive activation of GPR54 might lead to advancement of puberty onset, as it has been previously

demonstrated for other G protein-coupled receptors with key roles in the HPG axis, such as activating mutations of the LH receptor in boys [118]. However, such an etiopathogenic association is yet to be determined.

9. Conclusions

In the last 3 years, we have witnessed a substantial enlargement of our understanding of the physiological mechanisms responsible for the neuroendocrine control of the reproductive axis, and its eventual physiopathological alterations. Such a ‘revolution’ stemmed from the pioneering observations of the clinical consequences of genetic inactivation of the G protein-coupled receptor GPR54, which led (in a paradigmatic example of bedside-to-bench research) to the conduction of a comprehensive series of genetic, physiologic, pharmacological and clinical studies, that have set the contention that kisspeptins and GPR54 are not merely one more element in the cascade of signals controlling the gonadotropic axis, but instead operate as essential gatekeepers of GnRH function. Indeed, as schematically depicted in Fig. 7, KiSS-1 neurons in the forebrain are now recognized as primary afferents to GnRH neurons (where kisspeptins activate

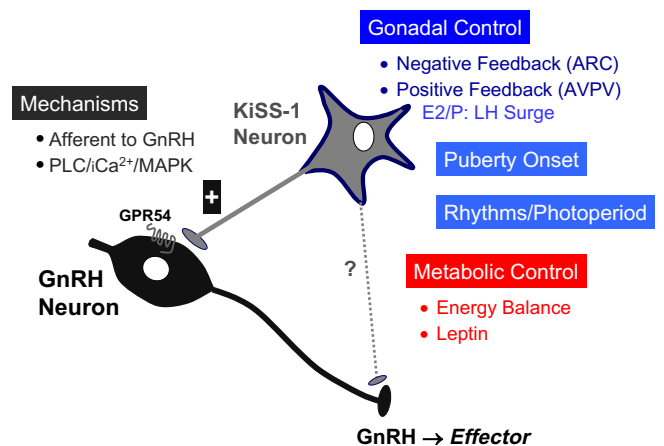


Fig. 7. Proposed model for the central role of KiSS-1 neurons in the integrated control of GnRH system and, hence, of the gonadotropic axis. KiSS-1 neurons, located in discrete hypothalamic nuclei, send projections to GnRH neurons, which express GPR54 and respond to kisspeptin with activation of early response gene expression and GnRH release; a response that involves the recruitment of PLC, mobilization of intracellular Ca^{2+} and activation of MAP kinases. Through such a central position, KiSS-1 neurons are likely to play a fundamental role in conveying the regulatory actions of gonadal sex steroids (estradiol: E2; progesterone: P) on GnRH neurons; both negative and positive feedback effects, via neuronal populations located at the arcuate nucleus (ARC) and the AVPV, respectively. In addition, compelling evidence has been presented for the essential roles of KiSS-1 neurons in the control puberty onset, the metabolic regulation of GnRH neurons and the photoperiodic modulation of reproduction (in seasonal breeders). Altogether, the experimental evidence available strongly supports a pivotal role of KiSS-1 neurons in the dynamic regulation of key aspects of reproductive function. For further details, see Section 9.

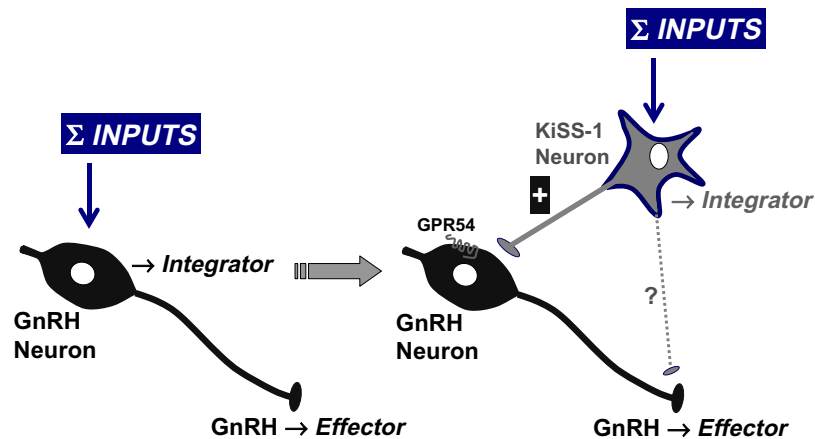


Fig. 8. Evolution of our understanding of the central neuroendocrine mechanisms controlling the gonadotropic axis. The original conception of GnRH serving as ultimate integrator of a diversity of regulators and final output pathway (*GnRH-centric model*; *left panel*) has been partially challenged by the recent identification of KiSS-1 neurons and the key roles of kisspeptins and GPR54 in the control of GnRH system. According to the tentative model depicted in the *right panel*, KiSS-1 neurons in the forebrain would operate as essential integrators of the information conveyed by different central and peripheral regulators of the gonadotropic axis. Conversely, GnRH neurons would play a more ‘passive’ role, acting as final transducers of the information relayed by the kisspeptin/GPR54 system. Admittedly, both models are not mutually exclusive, as GnRH neurons are likely to serve integratory functions themselves. For further details, see Section 9.

GPR54 signaling), and are known/suspected to receive and integrate critical signals for (i) the timing of puberty, (ii) the positive and negative feedback effects of sex steroids, (iii) the metabolic regulation of the gonadotropic axis (by leptin and eventually other factors), and (iv) the control of reproductive function by photoperiodic cues, among others. Overall, the available evidence strongly suggests that kisspeptin neurons do play a fundamental role in the integration of different afferents to the GnRH system; KiSS-1 neurons being ultimately responsible for driving the central activation of the HPG axis at different physiologic states. According to this tentative model, which is schematically presented in Fig. 8, the ‘center of gravity’ for the dynamic control of the reproductive axis would lie on the KiSS-1 neuron, on which the information conveyed by different central and peripheral regulators would converge and eventually integrate. Conversely, the GnRH neuron would play a more ‘passive’ role, acting as final transducer of the information relayed by the kisspeptin/GPR54 system. Admittedly, this hypothetical model, which is yet to be fully characterized, does not preclude the central function of GnRH as major output pathway for the central activation of the gonadotropic axis, neither does it rule out the possibility that GnRH neurons, whose dendrite morphology and afferents are considerably complex [19], may receive and/or integrate information independent of kisspeptin/GPR54 signaling, such as via glutamatergic and GABAergic neurotransmission [16,82]. In any event, such a model recognizes the fundamental roles of the elements of the KiSS-1/GPR54 system in the neuroendocrine control of Reproduction; a system whose neuroendocrine dimension remained unnoticed up to 3.5 years ago, and which is likely to attract an extraordinary attention among reproductive endocrinologists in the years to come.

Acknowledgments

The authors are indebted with the members of the research team at the Physiology Section of the University of Cordoba, as well as with F.F. Casanueva and staff from the Department of Physiology of the University of Santiago de Compostela, for superb collaboration in studies on neuroendocrine aspects of kisspeptin physiology and helpful discussions during preparation of this manuscript. The experimental work from the authors’ laboratory summarized in this review has been supported by Grants BFI 2002-00176 and BFU 2005-07446 from Ministerio de Educación y Ciencia, Spain, funds from Instituto de Salud Carlos III (Project PI042082), and EU research contract EDEN QLK4-CT-2002-00603.

References

- [1] S. Adachi, S. Yamada, Y. Takatsu, H. Matsui, M. Kinoshita, K. Takase, H. Sugiura, T. Ohtaki, H. Matsumoto, Y. Uenoyama, H. Tsukamura, K. Inoue, K.I. Maeda, Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats, *J. Reprod. Dev.* 53 (2007) 367–378.
- [2] A.C. Arai, Y.F. Xia, E. Suzuki, M. Kessler, O. Civelli, H.P. Nothacker, Cancer metastasis-suppressing peptide metastin upregulates excitatory synaptic transmission in hippocampal dentate granule cells, *J. Neurophysiol.* 94 (2005) 3648–3652.
- [3] J.A. Arreguin-Arevalo, C.A. Lents, T.A. Farmerie, T.M. Nett, C.M. Clay, KiSS-1 peptide induces release of LH by a direct effect on the hypothalamus of ovariectomized ewes, *Anim. Reprod. Sci.* 101 (2007) 265–275.
- [4] D.R. Beier, R.G. Dluhy, Bench and bedside—the G protein-coupled receptor GPR54 and puberty, *N. Engl. J. Med.* 349 (2003) 1589–1592.
- [5] M. Bilban, N. Ghaffari-Tabrizi, E. Hintermann, S. Bauer, S. Molzer, C. Zoratti, R. Malli, A. Sharabi, U. Hiden, W. Graier, M. Knofler, F. Andreae, O. Wagner, V. Quaranta, G. Desoye, Kisspeptin-10, a

- KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts, *J. Cell. Sci.* 117 (2004) 1319–1328.
- [6] G.C. Brailoiu, S.L. Dun, M. Ohsawa, D. Yin, J. Yang, J.K. Chang, E. Brailoiu, N.J. Dun, KiSS-1 expression and metastin-like immunoreactivity in the rat brain, *J. Comp. Neurol.* 481 (2005) 314–329.
- [7] S.M. Cadman, S.H. Kim, Y. Hu, D. Gonzalez-Martinez, P.M. Bouloux, Molecular pathogenesis of Kallmann's syndrome, *Horm. Res.* 67 (2006) 231–242.
- [8] F.F. Casanueva, C. Dieguez, Neuroendocrine regulation and actions of leptin, *Front. Neuroendocrinol.* 20 (1999) 317–363.
- [9] J.M. Castellano, M. Gaytan, J. Roa, E. Vigo, V.M. Navarro, C. Bellido, C. Dieguez, E. Aguilar, J.E. Sanchez-Criado, A. Pellicer, L. Pinilla, F. Gaytan, M. Tena-Sempere, Expression of KiSS-1 in rat ovary: putative local regulator of ovulation? *Endocrinology* 147 (2006) 4852–4862.
- [10] J.M. Castellano, V.M. Navarro, R. Fernandez-Fernandez, J.P. Castano, M.M. Malagon, E. Aguilar, C. Dieguez, P. Magni, L. Pinilla, M. Tena-Sempere, Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat, *Mol. Cell Endocrinol.* 257–258 (2006) 75–83.
- [11] J.M. Castellano, V.M. Navarro, R. Fernandez-Fernandez, R. Nogueiras, S. Tovar, J. Roa, M.J. Vazquez, E. Vigo, F.F. Casanueva, E. Aguilar, L. Pinilla, C. Dieguez, M. Tena-Sempere, Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition, *Endocrinology* 146 (2005) 3917–3925.
- [12] J.M. Castellano, V.M. Navarro, R. Fernandez-Fernandez, J. Roa, E. Vigo, R. Pineda, C. Dieguez, E. Aguilar, L. Pinilla, M. Tena-Sempere, Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropin responses by kisspeptin in streptozotocin-induced diabetic male rats, *Diabetes* 55 (2006) 2602–2610.
- [13] J.M. Castellano, V.M. Navarro, R. Fernandez-Fernandez, J. Roa, E. Vigo, R. Pineda, R.A. Steiner, E. Aguilar, L. Pinilla, M. Tena-Sempere, Effects of galanin-like peptide on luteinizing hormone secretion in the rat: sexually dimorphic responses and enhanced sensitivity at male puberty, *Am. J. Physiol. Endocrinol. Metab.* 291 (2006) E1281–E1289.
- [14] F. Cerrato, J. Shagoury, M. Kralickova, A. Dwyer, J. Falardeau, M. Ozata, G. Van Vliet, P. Bouloux, J.E. Hall, F.J. Hayes, N. Pitteloud, K.A. Martin, C. Welt, S.B. Seminara, Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism, *Eur. J. Endocrinol.* 155 (Suppl. 1) (2006) S3–S10.
- [15] I.J. Clarke, Two decades of measuring GnRH secretion, *Reprod. Suppl.* 59 (2002) 1–13.
- [16] J. Clarkson, A.E. Herbison, Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty, *Mol. Cell Endocrinol.* 254–255 (2006) 32–38.
- [17] J. Clarkson, A.E. Herbison, Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons, *Endocrinology* 147 (2006) 5817–5825.
- [18] W.H. Colledge, GPR54 and puberty, *Trends Endocrinol. Metab.* 15 (2004) 448–453.
- [19] E.C. Cottrell, R.E. Campbell, S.K. Han, A.E. Herbison, Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons, *Endocrinology* 147 (2006) 3652–3661.
- [20] H.J. Cho, J.S. Moon, J.S. Yang, J.I. Kim, J.Y. Seong, Molecular cloning of the bullfrog metastin receptor GPR54 (bfGPR54) and a functional analysis of bfGPR54 in comparison with rat GPR54, 23rd Conference of European Comparative Endocrinologists, Manchester, 2006, pp. 99.
- [21] X. d'Anglemont de Tassigny, L.A. Fagg, J.P. Dixon, K. Day, H.G. Leitch, A.G. Hendrick, D. Zahn, I. Franceschini, A. Caraty, M.B. Carlton, S.A. Aparicio, W.H. Colledge, Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene, *Proc. Natl. Acad. Sci. USA* 104 (2007) 10714–10719.
- [22] N. de Roux, E. Genin, J.C. Carel, F. Matsuda, J.L. Chaussain, E. Milgrom, Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54, *Proc. Natl. Acad. Sci. USA* 100 (2003) 10972–10976.
- [23] D.K. Dhar, H. Naora, H. Kubota, R. Maruyama, H. Yoshimura, Y. Tomomoto, M. Tachibana, T. Ono, H. Otani, N. Nagasue, Down-regulation of KiSS-1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma, *Int. J. Cancer* 111 (2004) 868–872.
- [24] W.S. Dhillon, O.B. Chaudhri, M. Patterson, E.L. Thompson, K.G. Murphy, M.K. Badman, B.M. McGowan, V. Amber, S. Patel, M.A. Ghatei, S.R. Bloom, Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males, *J. Clin. Endocrinol. Metab.* 90 (2005) 6609–6615.
- [25] W.S. Dhillon, P. Savage, K.G. Murphy, O.B. Chaudhri, M. Patterson, G.M. Nijher, V.M. Foggo, G.S. Dancy, H. Mitchell, M.J. Seckl, M.A. Ghatei, S.R. Bloom, Plasma kisspeptin is raised in patients with gestational trophoblastic neoplasia and falls during treatment, *Am. J. Physiol. Endocrinol. Metab.* 291 (2006) E878–E884.
- [26] K.M. Estrada, C.M. Clay, S. Pompolo, J.T. Smith, I.J. Clarke, Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotropin-releasing hormone/luteinizing hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback, *J. Neuroendocrinol.* 18 (2006) 806–809.
- [27] R. Fernandez-Fernandez, A.C. Martini, V.M. Navarro, J.M. Castellano, C. Dieguez, E. Aguilar, L. Pinilla, M. Tena-Sempere, Novel signals for the integration of energy balance and reproduction, *Mol. Cell Endocrinol.* 254–255 (2006) 127–132.
- [28] G. Fink, Neuroendocrine regulation of pituitary function: general principles, in: P.M. Conn, M.E. Freeman (Eds.), *Neuroendocrinology in Physiology and Medicine*, Humana Press, Totowa, New Jersey, 2000, pp. 107–134.
- [29] I. Franceschini, D. Lomet, M. Cateau, G. Delsol, Y. Tillet, A. Caraty, Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha, *Neurosci. Lett.* 401 (2006) 225–230.
- [30] S. Funes, J.A. Hedrick, G. Vassileva, L. Markowitz, S. Abbondanzo, A. Golovko, S. Yang, F.J. Monsma, E.L. Gustafson, The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system, *Biochem. Biophys. Res. Commun.* 312 (2003) 1357–1363.
- [31] M. Gaytan, J.M. Castellano, J. Roa, J.E. Sanchez-Criado, M. Tena-Sempere, F. Gaytan, Expression of KiSS-1 in rat oviduct: possible involvement in prevention of ectopic implantation? *Cell Tissue Res.* 329 (2007) 571–579.
- [32] M.L. Gottsch, D.K. Clifton, R.A. Steiner, Kisspeptin-GPR54 signaling in the neuroendocrine reproductive axis, *Mol. Cell Endocrinol.* 254–255 (2006) 91–96.
- [33] M.L. Gottsch, M.J. Cunningham, J.T. Smith, S.M. Popa, B.V. Acofido, W.F. Crowley, S. Seminara, D.K. Clifton, R.A. Steiner, A role for kisspeptins in the regulation of gonadotropin secretion in the mouse, *Endocrinology* 145 (2004) 4073–4077.
- [34] T.J. Greives, A.O. Mason, M.A. Scotti, J. Levine, E.D. Ketterson, L.J. Kriegsfeld, G.E. Demas, Environmental control of kisspeptin, in: implications for seasonal reproduction, *Endocrinology* 148 (2007) 1158–1166.
- [35] E. Gutierrez-Pascual, A.J. Martinez-Fuentes, L. Pinilla, M. Tena-Sempere, M.M. Malagon, J.P. Castano, Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs, and stimulation of luteinizing hormone and growth hormone secretion, *J. Neuroendocrinol.* 19 (2007) 521–530.
- [36] S.K. Han, M.L. Gottsch, K.J. Lee, S.M. Popa, J.T. Smith, S.K. Jakawich, D.K. Clifton, R.A. Steiner, A.E. Herbison, Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty, *J. Neurosci.* 25 (2005) 11349–11356.

- [37] J.F. Harms, D.R. Welch, M.E. Miele, KiSS1 metastasis suppression and emergent pathways, *Clin. Exp. Metastasis* 20 (2003) 11–18.
- [38] A.C. Hauge-Evans, C.C. Richardson, H.M. Milne, M.R. Christie, S.J. Persaud, P.M. Jones, A role for kisspeptin in islet function, *Diabetologia* 49 (2006) 2131–2135.
- [39] A.E. Herbison, J.R. Pape, New evidence for estrogen receptors in gonadotropin-releasing hormone neurons, *Front. Neuroendocrinol.* 22 (2001) 292–308.
- [40] U. Hiden, M. Bilban, M. Knofler, G. Desoye, Kisspeptins and the placenta: regulation of trophoblast invasion, *Rev. Endocr. Metab. Disord.* (2007).
- [41] Y. Horikoshi, H. Matsumoto, Y. Takatsu, T. Ohtaki, C. Kitada, S. Usuki, M. Fujino, Dramatic elevation of plasma metastatin concentrations in human pregnancy: metastatin as a novel placenta-derived hormone in humans, *J. Clin. Endocrinol. Metab.* 88 (2003) 914–919.
- [42] M. Ikeguchi, K. Yamaguchi, N. Kaibara, Clinical significance of the loss of KiSS-1 and orphan G-protein-coupled receptor (hOT7T175) gene expression in esophageal squamous cell carcinoma, *Clin. Cancer. Res.* 10 (2004) 1379–1383.
- [43] A. Iovane, C. Aumas, N. de Roux, New insights in the genetics of isolated hypogonadotropic hypogonadism, *Eur. J. Endocrinol.* 151 (Suppl. 3) (2004) U83–U88.
- [44] M.S. Irwig, G.S. Fraley, J.T. Smith, B.V. Acohido, S.M. Popa, M.J. Cunningham, M.L. Gottsch, D.K. Clifton, R.A. Steiner, Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat, *Neuroendocrinology* 80 (2004) 264–272.
- [45] Y. Jiang, M. Berk, L.S. Singh, H. Tan, L. Yin, C.T. Powell, Y. Xu, KiSS1 suppresses metastasis in human ovarian cancer via inhibition of protein kinase C alpha, *Clin. Exp. Metastasis* 22 (2005) 369–376.
- [46] A.S. Kauffman, M.L. Gottsch, J. Roa, A.C. Byquist, A. Crown, D.K. Clifton, G.E. Hoffman, R.A. Steiner, M. Tena-Sempere, Sexual differentiation of kiss1 gene expression in the brain of the rat, *Endocrinology* 148 (2007) 1774–1783.
- [47] M. Kinoshita, H. Tsukamura, S. Adachi, H. Matsui, Y. Uenoyama, K. Iwata, S. Yamada, K. Inoue, T. Ohtaki, H. Matsumoto, K. Maeda, Involvement of central metastatin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats, *Endocrinology* 146 (2005) 4431–4436.
- [48] M. Kotani, M. Dethoux, A. Vandenbogaerde, D. Communi, J.M. Vanderwinden, E. Le Poul, S. Brezillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S.N. Schiffmann, G. Vassart, M. Parmentier, The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54, *J. Biol. Chem.* 276 (2001) 34631–34636.
- [49] T.R. Kumar, What have we learned about gonadotropin function from gonadotropin subunit and receptor knockout mice? *Reproduction* 130 (2005) 293–302.
- [50] F. Lanfranco, J. Gromoll, S. von Eckardstein, E.M. Herding, E. Nieschlag, M. Simoni, Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism, *Eur. J. Endocrinol.* 153 (2005) 845–852.
- [51] D.K. Lee, T. Nguyen, G.P. O'Neill, R. Cheng, Y. Liu, A.D. Howard, N. Coulombe, C.P. Tan, A.T. Tang-Nguyen, S.R. George, B.F. O'Dowd, Discovery of a receptor related to the galanin receptors, *FEBS Lett.* 446 (1999) 103–107.
- [52] J.H. Lee, M.E. Miele, D.J. Hicks, K.K. Phillips, J.M. Trent, B.E. Weissman, D.R. Welch, KiSS-1, a novel human malignant melanoma metastasis-suppressor gene, *J. Natl. Cancer Inst.* 88 (1996) 1731–1737.
- [53] J.H. Lee, D.R. Welch, Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display, *Int. J. Cancer* 71 (1997) 1035–1044.
- [54] J.H. Lee, D.R. Welch, Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1, *Cancer Res.* 57 (1997) 2384–2387.
- [55] J.E. Levine, P.E. Chappell, J.S. Schneider, N.C. Sleiter, M. Szabo, Progesterone receptors as neuroendocrine integrators, *Front. Neuroendocrinol.* 22 (2001) 69–106.
- [56] R.M. Luque, R.D. Kineman, M. Tena-Sempere, Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: Analyses using mouse models and a cell line, *Endocrinology* (2007), doi:10.1210/en.2007-0500.
- [57] M.A. Magiakou, G. Mastorakos, E. Webster, G.P. Chrousos, The hypothalamic-pituitary-adrenal axis and the female reproductive system, *Ann N. Y. Acad. Sci.* 816 (1997) 42–56.
- [58] T.A. Martin, G. Watkins, W.G. Jiang, KiSS-1 expression in human breast cancer, *Clin. Exp. Metastasis* 22 (2005) 503–511.
- [59] T. Masui, R. Doi, T. Mori, E. Toyoda, M. Koizumi, K. Kami, D. Ito, S.C. Peiper, J.R. Broach, S. Oishi, A. Niida, N. Fujii, M. Imamura, Metastatin and its variant forms suppress migration of pancreatic cancer cells, *Biochem. Biophys. Res. Commun.* 315 (2004) 85–92.
- [60] H. Matsui, Y. Takatsu, S. Kumano, H. Matsumoto, T. Ohtaki, Peripheral administration of metastatin induces marked gonadotropin release and ovulation in the rat, *Biochem. Biophys. Res. Commun.* 320 (2004) 383–388.
- [61] E.J. Mead, J.J. Maguire, R.E. Kuc, A.P. Davenport, Kisspeptins are novel potent vasoconstrictors in humans, with a discrete localization of their receptor, G protein-coupled receptor 54, to atherosclerosis-prone vessels, *Endocrinology* 148 (2007) 140–147.
- [62] S. Messenger, Kisspeptin and its receptor: new gatekeepers of puberty, *J. Neuroendocrinol.* 17 (2005) 687–688.
- [63] S. Messenger, E.E. Chatzidaki, D. Ma, A.G. Hendrick, D. Zahn, J. Dixon, R.R. Thresher, I. Malinge, D. Lomet, M.B. Carlton, W.H. Colledge, A. Caraty, S.A. Aparicio, Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54, *Proc. Natl. Acad. Sci. USA* 102 (2005) 1761–1766.
- [64] D.C. Mitchell, M. Abdelrahim, J. Weng, L.J. Stafford, S. Safe, M. Bar-Eli, M. Liu, Regulation of KiSS-1 metastasis suppressor gene expression in breast cancer cells by direct interaction of transcription factors activator protein-2alpha and specificity protein-1, *J. Biol. Chem.* 281 (2006) 51–58.
- [65] J.S. Mohamed, A.D. Benninghoff, G.J. Holt, I.A. Khan, Developmental expression of the G protein-coupled receptor 54 and three GnRH mRNAs in the teleost fish cobia, *J. Mol. Endocrinol.* 38 (2007) 235–244.
- [66] J.A. Morris, C.L. Jordan, S.M. Breedlove, Sexual differentiation of the vertebrate nervous system, *Nat. Neuroscience* 7 (2004) 1034–1039.
- [67] A.I. Muir, L. Chamberlain, N.A. Elshourbagy, D. Michalovich, D.J. Moore, A. Calamari, P.G. Szekeres, H.M. Sarau, J.K. Chambers, P. Murdock, K. Steplewski, U. Shabon, J.E. Miller, S.E. Middleton, J.G. Darker, C.G. Larminie, S. Wilson, D.J. Bergsma, P. Emson, R. Faull, K.L. Philpott, D.C. Harrison, AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1, *J. Biol. Chem.* 276 (2001) 28969–28975.
- [68] K.T. Nash, P.A. Phadke, J.M. Navenot, D.R. Hurst, M.A. Accavitti-Loper, E. Sztul, K.S. Vaidya, A.R. Frost, J.C. Kappes, S.C. Peiper, D.R. Welch, Requirement of KiSS1 secretion for multiple organ metastasis suppression and maintenance of tumor dormancy, *J. Natl. Cancer Inst.* 99 (2007) 309–321.
- [69] V.M. Navarro, J.M. Castellano, R. Fernandez-Fernandez, M.L. Barreiro, J. Roa, J.E. Sanchez-Criado, E. Aguilar, C. Dieguez, L. Pinilla, M. Tena-Sempere, Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide, *Endocrinology* 145 (2004) 4565–4574.
- [70] V.M. Navarro, J.M. Castellano, R. Fernandez-Fernandez, S. Tovar, J. Roa, A. Mayen, M.L. Barreiro, F.F. Casanueva, E. Aguilar, C. Dieguez, L. Pinilla, M. Tena-Sempere, Effects of KiSS-1 peptide, the natural ligand of GPR54, on follicle-stimulating hormone secretion in the rat, *Endocrinology* 146 (2005) 1689–1697.

- [71] V.M. Navarro, J.M. Castellano, R. Fernandez-Fernandez, S. Tovar, J. Roa, A. Mayen, R. Nogueiras, M.J. Vazquez, M.L. Barreiro, P. Magni, E. Aguilar, C. Dieguez, L. Pinilla, M. Tena-Sempere, Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54, *Endocrinology* 146 (2005) 156–163.
- [72] V.M. Navarro, R. Fernandez-Fernandez, J.M. Castellano, J. Roa, A. Mayen, M.L. Barreiro, F. Gaytan, E. Aguilar, L. Pinilla, C. Dieguez, M. Tena-Sempere, Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54, *J. Physiol.* 561 (2004) 379–386.
- [73] S.J. Nazian, Role of metastin in the release of gonadotropin-releasing hormone from the hypothalamus of the male rat, *J. Androl.* 27 (2006) 444–449.
- [74] A. Niida, Z. Wang, K. Tomita, S. Oishi, H. Tamamura, A. Otaka, J.M. Navenot, J.R. Broach, S.C. Peiper, N. Fujii, Design and synthesis of downsized metastin (45–54) analogs with maintenance of high GPR54 agonistic activity, *Bioorg. Med. Chem. Lett.* 16 (2006) 134–137.
- [75] J.N. Nocillado, B. Levavi-Sivan, F. Carrick, A. Elizur, Temporal expression of G-protein-coupled receptor 54 (GPR54), gonadotropin-releasing hormones (GnRH), and dopamine receptor D2 (drd2) in pubertal female grey mullet, *Mugil cephalus*, *Gen. Comp. Endocrinol.* 150 (2007) 278–287.
- [76] R.L. Norman, H.G. Spies, Cyclic ovarian function in a male macaque: additional evidence for a lack of sexual differentiation in the physiological mechanisms that regulate the cyclic release of gonadotropins in primates, *Endocrinology* 118 (1986) 2608–2610.
- [77] T. Ohtaki, Y. Shintani, S. Honda, H. Matsumoto, A. Hori, K. Kanehashi, Y. Terao, S. Kumano, Y. Takatsu, Y. Masuda, Y. Ishibashi, T. Watanabe, M. Asada, T. Yamada, M. Suenaga, C. Kitada, S. Usuki, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor, *Nature* 411 (2001) 613–617.
- [78] S.R. Ojeda, A. Lomniczi, C. Mastronardi, S. Heger, C. Roth, A.S. Parent, V. Matagne, A.E. Mungenast, Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology* 147 (2006) 1166–1174.
- [79] S.R. Ojeda, Y.J. Ma, B.J. Lee, V. Prevot, Glia-to-neuron signaling and the neuroendocrine control of female puberty, *Recent Prog. Horm. Res.* 55 (2000) 197–223 (Discussion 223–194).
- [80] S.R. Ojeda, M.K. Skinner, Puberty in the rat, in: J.D. Neill (Ed.), *The Physiology of Reproduction*, Academic Press, Elsevier, San Diego, 2005, pp. 2061–2126.
- [81] M.J. Orsini, M.A. Klein, M.P. Beavers, P.J. Connolly, S.A. Middleton, K.H. Mayo, Metastin (KiSS-1) mimetics identified from peptide structure–activity relationship-derived pharmacophores and directed small molecule database screening, *J. Med. Chem.* 50 (2007) 462–471.
- [82] E.N. Ottem, J.G. Godwin, S.L. Petersen, Glutamatergic signaling through the *N*-methyl-D-aspartate receptor directly activates medial subpopulations of luteinizing hormone-releasing hormone (LHRH) neurons, but does not appear to mediate the effects of estradiol on LHRH gene expression, *Endocrinology* 143 (2002) 4837–4845.
- [83] J.C. Pallais, Y. Bo-Abbas, N. Pitteloud, W.F. Crowley Jr., S.B. Seminara, Neuroendocrine, gonadal, placental, and obstetric phenotypes in patients with IHH and mutations in the G-protein coupled receptor, GPR54, *Mol. Cell Endocrinol.* 254–255 (2006) 70–77.
- [84] A.S. Parent, G. Teilmann, A. Juul, N.E. Skakkebaek, J. Toppari, J.P. Bourguignon, The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration, *Endocr. Rev.* 24 (2003) 668–693.
- [85] I.S. Parhar, S. Ogawa, Y. Sakuma, Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish, *Endocrinology* 145 (2004) 3613–3618.
- [86] M. Patterson, K.G. Murphy, E.L. Thompson, S. Patel, M.A. Ghatei, S.R. Bloom, Administration of kisspeptin-54 into discrete regions of the hypothalamus potently increases plasma luteinizing hormone and testosterone in male adult rats, *J. Neuroendocrinol.* 18 (2006) 349–354.
- [87] L. Ping, V.B. Mahesh, G.K. Bhat, D.W. Brann, Regulation of gonadotropin-releasing hormone and luteinizing hormone secretion by AMPA receptors. Evidence for a physiological role of AMPA receptors in the steroid-induced luteinizing hormone surge, *Neuroendocrinology* 66 (1997) 246–253.
- [88] T.M. Plant, The role of KiSS-1 in the regulation of puberty in higher primates, *Eur. J. Endocrinol.* 155 (Suppl. 1) (2006) S11–S16.
- [89] T.M. Plant, S. Ramaswamy, M.J. Dipietro, Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges, *Endocrinology* 147 (2006) 1007–1013.
- [90] C.R. Pohl, L.R. Lee, M.S. Smith, Qualitative changes in luteinizing hormone and prolactin responses to *N*-methyl-aspartic acid during lactation in the rat, *Endocrinology* 124 (1989) 1905–1911.
- [91] S. Pompolo, A. Pereira, K.M. Estrada, I.J. Clarke, Colocalization of kisspeptin and gonadotropin-releasing hormone in the ovine brain, *Endocrinology* 147 (2006) 804–810.
- [92] S. Ramaswamy, S.B. Seminara, C.R. Pohl, M.J. Dipietro, W.F. Crowley Jr., T.M. Plant, Effect of continuous iv administration of human metastin 45–54 on the neuroendocrine activity of the hypothalamic-pituitary-testicular axis in the adult male rhesus monkey (*Macaca mulatta*), *Endocrinology* 148 (2007) 3364–3370.
- [93] F.G. Revel, M. Saboureau, M. Masson-Pevet, P. Pevet, J.D. Mikkelsen, V. Simonneaux, KiSS-1: a likely candidate for the photoperiodic control of reproduction in seasonal breeders, *Chronobiol. Int.* 23 (2006) 277–287.
- [94] F.G. Revel, M. Saboureau, M. Masson-Pevet, P. Pevet, J.D. Mikkelsen, V. Simonneaux, Kisspeptin mediates the photoperiodic control of reproduction in hamsters, *Curr. Biol.* 16 (2006) 1730–1735.
- [95] F.I. Reyes, J.S. Winter, C. Faiman, Pituitary gonadotropin function during human pregnancy: serum FSH and LH levels before and after LHRH administration, *J. Clin. Endocrinol. Metab.* 42 (1976) 590–592.
- [96] J. Roa, M. Tena-Sempere, KiSS-1 system and reproduction: comparative aspects and roles in the control of female gonadotropic axis in mammals, *Gen. Comp. Endocrinol.* 153 (2007) 132–140.
- [97] J. Roa, E. Vigo, J.M. Castellano, V.M. Navarro, R. Fernandez-Fernandez, F.F. Casanueva, C. Dieguez, E. Aguilar, L. Pinilla, M. Tena-Sempere, Hypothalamic expression of KiSS-1 system and gonadotropin-releasing effects of kisspeptin in different reproductive states of the female rat, *Endocrinology* 147 (2006) 2864–2878.
- [98] A.M. Rometo, S.J. Krajewski, M.L. Voytko, N.E. Rance, Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys, *J. Clin. Endocrinol. Metab.* 92 (2007) 2744–2750.
- [99] M. Sanchez-Carbayo, P. Capodiceci, C. Cordon-Cardo, Tumor suppressor role of KiSS-1 in bladder cancer: loss of KiSS-1 expression is associated with bladder cancer progression and clinical outcome, *Am. J. Pathol.* 162 (2003) 609–617.
- [100] K. Schmid, X. Wang, A. Haitel, W. Sieghart, M. Peck-Radosavljevic, M. Bodingbauer, S. Rasoul-Rockenschaub, F. Wrba, KiSS-1 overexpression as an independent prognostic marker in hepatocellular carcinoma: an immunohistochemical study, *Virchows Arch.* (2007).
- [101] N.B. Schwartz, Neuroendocrine regulation of reproductive cyclicity, in: P.M. Conn, M.E. Freeman (Eds.), *Neuroendocrinology in Physiology and Medicine*, Humana Press, Totowa, New Jersey, 2000, pp. 135–146.
- [102] S.B. Seminara, Metastin and its G protein-coupled receptor, GPR54: critical pathway modulating GnRH secretion, *Front. Neuroendocrinol.* 26 (2005) 131–138.
- [103] S.B. Seminara, M.J. Dipietro, S. Ramaswamy, W.F. Crowley Jr., T.M. Plant, Continuous human metastin 45–54 infusion desensitizes

- G protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male Rhesus monkey (*Macaca mulatta*): a finding with therapeutic implications, *Endocrinology* 147 (2006) 2122–2126.
- [104] S.B. Seminara, S. Messenger, E.E. Chatzidaki, R.R. Thresher, J.S. Acierno Jr., J.K. Shagoury, Y. Bo-Abbas, W. Kuohung, K.M. Schwino, A.G. Hendrick, D. Zahn, J. Dixon, U.B. Kaiser, S.A. Slangenaupt, J.F. Gusella, S. O'Rahilly, M.B. Carlton, W.F. Crowley Jr., S.A. Aparicio, W.H. Colledge, The GPR54 gene as a regulator of puberty, *N. Engl. J. Med.* 349 (2003) 1614–1627.
- [105] R.K. Semple, J.C. Achermann, J. Ellery, I.S. Farooqi, F.E. Karet, R.G. Stanhope, S. O'Rahilly, S.A. Aparicio, Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism, *J. Clin. Endocrinol. Metab.* 90 (2005) 1849–1855.
- [106] M. Shahab, C. Mastronardi, S.B. Seminara, W.F. Crowley, S.R. Ojeda, T.M. Plant, Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates, *Proc. Natl. Acad. Sci. USA* 102 (2005) 2129–2134.
- [107] M. Shibata, R.L. Friedman, S. Ramaswamy, T.M. Plant, Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (*Macaca mulatta*), *J. Neuroendocrinol.* 19 (2007) 432–438.
- [108] J.T. Smith, B.V. Acohido, D.K. Clifton, R.A. Steiner, KiSS-1 neurones are direct targets for leptin in the ob/ob mouse, *J. Neuroendocrinol.* 18 (2006) 298–303.
- [109] J.T. Smith, C.M. Clay, A. Caraty, I.J. Clarke, KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season, *Endocrinology* 148 (2007) 1150–1157.
- [110] J.T. Smith, M.J. Cunningham, E.F. Rissman, D.K. Clifton, R.A. Steiner, Regulation of Kiss1 gene expression in the brain of the female mouse, *Endocrinology* 146 (2005) 3686–3692.
- [111] J.T. Smith, H.M. Dungan, E.A. Stoll, M.L. Gottsch, R.E. Braun, S.M. Eacker, D.K. Clifton, R.A. Steiner, Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse, *Endocrinology* 146 (2005) 2976–2984.
- [112] J.T. Smith, S.M. Popa, D.K. Clifton, G.E. Hoffman, R.A. Steiner, Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge, *J. Neurosci.* 26 (2006) 6687–6694.
- [113] M.S. Smith, K.L. Grove, Integration of the regulation of reproductive function and energy balance: lactation as a model, *Front. Neuroendocrinol.* 23 (2002) 225–256.
- [114] M. Tena-Sempere, Hypothalamic KiSS-1: the missing link in gonadotropin feedback control? *Endocrinology* 146 (2005) 3683–3685.
- [115] M. Tena-Sempere, GPR54 and kisspeptin in reproduction, *Hum. Reprod. Update* 12 (2006) 631–639.
- [116] M. Tena-Sempere, KiSS-1 and reproduction: focus on its role in the metabolic regulation of fertility, *Neuroendocrinology* 83 (2006) 275–281.
- [117] M. Tena-Sempere, The roles of kisspeptins and G protein-coupled receptor-54 in pubertal development, *Curr. Opin. Pediatr.* 18 (2006) 442–447.
- [118] M. Tena-Sempere, I. Huhtaniemi, Gonadotropins and gonadotropin receptors, in: B.C.J.M. Fauser (Ed.), *Reproductive Medicine—Molecular, Cellular and Genetic Fundamentals*, Springer, New York, 2003, pp. 225–244.
- [119] M. Tena-Sempere, L. Pinilla, L.C. Gonzalez, E. Aguilar, Reproductive disruption by exposure to exogenous estrogenic compounds during sex differentiation: lessons from the neonatally estrogenized male rat, *Curr. Top. Steroid Res.* 3 (2000) 23–37.
- [120] Y. Tenenbaum-Rakover, M. Commenges-Ducos, A. Iovane, C. Aumas, O. Admoni, N. de Roux, Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54, *J. Clin. Endocrinol. Metab.* 92 (2007) 1137–1144.
- [121] Y. Terao, S. Kumano, Y. Takatsu, M. Hattori, A. Nishimura, T. Ohtaki, Y. Shintani, Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta, *Biochim. Biophys. Acta* 1678 (2004) 102–110.
- [122] J.C. Thiery, P. Chemineau, X. Hernandez, M. Migaud, B. Malpoux, Neuroendocrine interactions and seasonality, *Domest. Anim. Endocrinol.* 23 (2002) 87–100.
- [123] E.L. Thompson, M. Patterson, K.G. Murphy, K.L. Smith, W.S. Dhillon, J.F. Todd, M.A. Ghatei, S.R. Bloom, Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis, *J. Neuroendocrinol.* 16 (2004) 850–858.
- [124] K. Tomita, T. Narumi, A. Niida, S. Oishi, H. Ohno, N. Fujii, Fmoc-based solid-phase synthesis of GPR54-agonistic pentapeptide derivatives containing alkene- and fluoroalkene-dipeptide isosteres, *Bio-polymers* 88 (2007) 272–278.
- [125] K. Tomita, A. Niida, S. Oishi, H. Ohno, J. Cluzeau, J.M. Navenot, Z.X. Wang, S.C. Peiper, N. Fujii, Structure–activity relationship study on small peptidic GPR54 agonists, *Bioorg. Med. Chem.* 14 (2006) 7595–7603.
- [126] S. Tovar, M.J. Vazquez, V.M. Navarro, R. Fernandez-Fernandez, J.M. Castellano, E. Vigo, J. Roa, F.F. Casanueva, E. Aguilar, L. Pinilla, C. Dieguez, M. Tena-Sempere, Effects of single or repeated intravenous administration of kisspeptin upon dynamic LH secretion in conscious male rats, *Endocrinology* 147 (2006) 2696–2704.
- [127] A. West, P.J. Vojta, D.R. Welch, B.E. Weissman, Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1), *Genomics* 54 (1998) 145–148.
- [128] T.M. Wintermantel, R.E. Campbell, R. Porteous, D. Bock, H.J. Grone, M.G. Todman, K.S. Korach, E. Greiner, C.A. Perez, G. Schutz, A.E. Herbison, Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility, *Neuron* 52 (2006) 271–280.
- [129] S. Yamada, Y. Uenoyama, M. Kinoshita, K. Iwata, K. Takase, H. Matsui, S. Adachi, K. Inoue, K.I. Maeda, H. Tsukamura, Inhibition of metastin (kisspeptin-54)-GPR 54 signaling in the arcuate nucleus-median eminence region during lactation in rats, *Endocrinology* 148 (2007) 2226–2232.