REVIEW

ENDOCRINOLOGY AND ADOLESCENCE

Deciphering puberty: novel partners, novel mechanisms

Manuel Tena-Sempere^{1,2,3}

¹Department of Cell Biology, Physiology and Immunology, Faculty of Medicine, University of Córdoba, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain, ²CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, 14004 Córdoba, Spain and ³Instituto Maimónides de Investigaciones Biomédicas (IMIBIC)/Hospital Universitario Reina Sofía, 14004 Córdoba, Spain

(Correspondence should be addressed to M Tena-Sempere at Department of Cell Biology, Physiology and Immunology, Faculty of Medicine, University of Córdoba; Email: f11tesem@uco.es)

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Abstract

Puberty is a fascinating developmental phase that involves the attainment of reproductive capacity and the completion of sexual and somatic maturation. As a life-changing event, puberty onset is precisely controlled by interconnected regulatory pathways that are sensitive to numerous endogenous signals and environmental cues. The mechanisms of normal puberty and its potential deviations have been thoroughly studied in humans and model species. Yet, characterization of the neurobiological basis of puberty is still incomplete. Progress on this front is not only relevant from a physiological perspective but would also help to unravel the underlying causes for the observed changes in the timing of puberty in humans, with a trend for earlier puberty onset, especially in girls. In this review, we will provide a synoptic overview of some recent developments in the field that have deepened our understanding of the neuroendocrine and molecular basis for the control of puberty onset. These include not only the demonstration of the involvement of the hypothalamic Kiss1 system in the control of puberty and its modulation by metabolic cues but also the identification of the roles of other neuropeptide pathways and molecular mediators in the regulation of puberty. In addition, the potential contribution of novel regulatory mechanisms, such as epigenetics, in the central control of puberty will be briefly discussed. Characterization of these novel players and regulatory mechanisms will improve our understanding of the basis of normal puberty and its eventual alterations in various pathological conditions.

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Neurobiology of puberty: single trigger or multiple regulatory networks?

Puberty is a key developmental phenomenon in sexual and somatic maturation (1, 2, 3). At puberty, reproductive capacity is achieved and phenotypic sexual maturity attained. In addition, important growth, behavioral, and psychological changes occur at puberty, thus allowing the acquisition of a complete adult phenotype. The timing of puberty is the final result of the interplay between strong genetic determinants (4) and a large number of regulators, which include different endogenous factors and environmental signals, from nutrient availability to photic cues (2). Of note, these interactions are not restricted to the pubertal transition but are rather initiated at very early developmental stages, so that puberty can be considered the end point (and sensor) of a maturational continuum shaped by the dynamic interactions of genes and environment during prenatal and postnatal

development (5). Because of its relevance in the lifetime of any individual, the physiology of puberty and the basis for its eventual deviations have been thoroughly analyzed in numerous species, through different experimental and methodological approaches. Yet, despite the considerable progress in the field, essential aspects of puberty and its underlying mechanisms remain ill defined.

From a neurobiological perspective, a hallmark of puberty onset is the heightening of the neurosecretory activity of GnRH neurons in the basal forebrain, which in turn maximally activates the gonadotropic axis to drive complete gonadal maturation and adult function (3, 6). Episodic secretion of GnRH, which is mandatory for proper stimulation of gonadotropin release and, hence, gonadal function, is the result of the interplay between the intrinsic oscillatory nature of GnRH neurons and a wide array of excitatory and inhibitory afferents that integrate at the so-called GnRH pulse generator (1). On the latter, it has been documented

that pubertal changes in pulsatile GnRH secretion are caused by the concerted modifications in trans-synaptic and glial inputs to the GnRH neuronal network (1, 3, 6). Within this complex circuitry, neuronal afferents to GnRH neurons likely operate as ultimately responsible for the triggering of puberty. While the nature of such a network of neuronal transmitters has been partially elucidated in recent years, our understanding of the whole set of regulatory signals that project onto GnRH neurons, as well as of their effects and major mechanisms of action, remains incomplete. Similarly, the molecular mechanisms whereby these signals are integrated at the level of GnRH neurons to define specific patterns of pulsatile secretion are still poorly understood.

Of note, system biology approaches have recently allowed to identify sets of genes/proteins that become activated at the time of puberty. This has led to the proposal that, rather than the consequence of the action of a single trigger (which has been long sought by generations of neuroendocrinologists), puberty is likely the end point of the concerted and hierarchical activation/inactivation of excitatory and inhibitory networks (3, 6), whose timed regulation would require precise and multifaceted control mechanisms that are yet to be fully exposed. Recognition of such complexity, and our as yet limited knowledge of this essential phenomenon in the life course of any individual, has fueled active research, both clinical and experimental, in this area of biomedicine.

As a result of these activities, during recent years, we have witnessed a substantial expansion of our knowledge of the physiological basis of puberty. Without any doubt, a major development in the field came with the identification of kisspeptins (Kp) as essential gatekeepers of puberty, a role that has drawn considerable attention in recent years (7, 8, 9, 10), and will be discussed herein, especially in light of recent developments that seem to challenge the dogma of an indispensable role of kisspeptins in puberty. In addition, attention will be paid in this review to summarize some illustrative examples of the identification of additional central transmitters and regulatory mechanisms (putatively) involved in the control of puberty and its modulation by the metabolic status. In doing so, we intend to provide a 'flavor' of the active research work conducted in this area in recent years, activities that will be crucial to refine our understanding of how puberty is controlled (and eventually altered) in humans and other mammals.

Kisspeptins: major gatekeepers of puberty

Among the different trans-synaptic regulators of GnRH neurons, Kp have drawn substantial attention in recent years, as essential gatekeepers of puberty onset and reproductive function (7, 11, 12). In fact,

identification of Kp is now considered as one of the major breakthroughs in reproductive biology since the isolation of GnRH back in the early 1970s (7, 11). As extensively reviewed elsewhere, kisspeptins, which include Kp-54 and Kp-10, are a family of structurally related peptides, encoded by the *Kiss1* gene, that act via the G protein-coupled receptor *Gpr54* (also termed *Kiss1R*) (7, 8, 10). The first evidence about the reproductive roles of kisspeptins and *Gpr54* dates back to late 2003, when inactivating mutations of the receptor were described in patients with hypogonadotropic hypogonadism (HH), a pathological condition of impuberism and infertility of central origin (13, 14). Very recently, the first inactivating mutation of the *KISS1* gene in humans with HH has been reported (15). Mice engineered to lack functional *Gpr54* or *Kiss1* have been shown to be a phenocopy of affected humans (13). Importantly, initial analyses in patients and mice with null mutations of *Gpr54* suggested that kisspeptins are key elements in the control of GnRH secretion (7, 16), a contention that was later substantiated by the demonstration of expression of *Gpr54* in GnRH neurons and by the proven ability of kisspeptins to robustly activate GnRH neurons and GnRH secretion in different species (7, 11, 12).

Detailed analyses have surfaced the patterns of anatomical distribution of the hypothalamic *Kiss1* neurons with important roles in the control of the GnRH system. These patterns have been well characterized in rodents, where two prominent populations of *Kiss1* neurons have been identified: one located in the arcuate nucleus (ARC) and the other in the rostral periventricular area of the third ventricle (RP3V) (7). In other mammals, including primates, an abundant set of *Kiss1* neurons is present in the ARC/infundibular region, whereas the existence of a population equivalent to the group of RP3V *Kiss1* neurons in rodents is still under debate (7, 10, 12). Despite their common capacity to produce kisspeptins, ARC and RP3V *Kiss1* neurons respond differently to key regulators (e.g. sex steroids) and appear to play rather different roles in the control of various aspects of reproduction (17). The functional features and molecular mechanisms responsible for the divergent regulation of *Kiss1* neurons in different hypothalamic sites are yet to be deciphered.

One of the facets of the *Kiss1* system that has attracted more attention is its potential implication in the control of puberty (18, 19). This was suggested by original observations of absence of puberty in humans and mice with genetic inactivation of *Gpr54* (7) and supported by recent findings of impuberism in humans with null mutations of *KISS1* (15). However, data from models of congenital inactivation of *Gpr54* or *Kiss1* provide little insight into the mechanisms whereby kisspeptins participate in the activational control of puberty. These mechanisms, however, have been partially disclosed recently by a combination of expression and functional analyses, including those of

our group, that documented the complex pattern of developmental activation of Kiss1 neurons along puberty, which includes the following (18, 20): i) an increase in endogenous kisspeptin tone, sufficient *per se* to fully activate the GnRH/gonadotropin axis; 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 and resistance to desensitization to continuous kisspeptin stimulation; and iv) an increase in the number of kisspeptin neurons and their projections to GnRH neurons. In good agreement, studies from our group have demonstrated that pharmacological blockade of Gpr54 is able to delay puberty onset in rodents (19).

Despite this compelling evidence, some aspects of the pubertal roles of kisspeptins remain obscure. For instance, the driving signals and molecular mechanisms underlying the activational program of Kiss1 neurons during puberty remain unknown, although studies in mice have suggested that estrogens are responsible for the pubertal expansion of the Kiss1 neuronal populations (10, 18); whether the same mechanism applies in humans is yet to be defined. Moreover, a recent report challenged the consensus view for an essential role of kisspeptins in puberty onset in rodents by showing that fertility can be attained even after congenital ablation of Kiss1 neurons in female mice (21). Of note, however, timed elimination of

Kiss1 neurons during the early juvenile period did perturb pubertal maturation and induced infertility (21). One possible explanation for these perplexing observations is that a residual set of Kiss1 neurons after congenital ablation might be sufficient to drive puberty onset, due to compensatory mechanisms, as has been previously suggested for GnRH neurons as well (22). Altogether, these findings illustrate the complex developmental roles of Kiss1 neurons that might be compensated at early maturational stages but are nonetheless indispensable for the activational mechanisms leading to puberty onset. Of note, a number of expression analyses have suggested differential roles for the ARC and RP3V Kiss1 neurons in the timing of puberty, but the nature of such differential actions and their eventual regulatory mechanisms are yet to be elucidated (18, 20). A synoptic compilation of the supportive evidence, challenging data, and open questions regarding the role of Kiss1 pathways in the central control of puberty is presented in Table 1.

Interactive partners of Kiss1: roles of neurokinin B in the control of puberty

A facet of the Kiss1 system that has drawn considerable interest recently has been the identification of regulatory signals and interactive partners of kisspeptins.

Table 1 Synoptic compilation of the supporting experimental evidence, challenging data, and open questions regarding the role of Kiss1 pathways in the central control of puberty. For further details, see sections ‘Kisspeptins: major gatekeepers of puberty’ and ‘Translational medicine and the control of puberty: preclinical models and species differences’.

	Species
Supporting evidence	
Lack of kisspeptin signaling (e.g. null mutations of <i>Kiss1</i> or <i>Gpr54</i>) prevents/delays puberty onset	Human, mouse (10 ^a , 13, 14, 15)
Ablation of Kiss1 neurons during the late infantile period perturbs puberty onset and prevents attainment of fertility	Mouse (21)
Transient blockade (antagonism) of kisspeptin signaling delays puberty onset	Rat (19)
Hypothalamic expression of <i>Kiss1</i> mRNA and/or the number of Kiss1 neurons and their projections to GnRH neurons increase during pubertal maturation	Monkey, rat, mouse, and sheep (9 ^a , 10 ^a , 42, 100)
A rise in the pulsatile release of kisspeptin in the median eminence takes place at puberty	Monkey (101)
Exogenous administration of kisspeptins advances the onset of puberty and activates the pulsatile release of GnRH	Rat and monkey (10 ^a , 18 ^a)
Responsiveness to kisspeptins increases, while kisspeptin desensitization decreases, during pubertal maturation	Rat and mouse (10 ^a , 18 ^a)
Conditions of metabolic stress that alter puberty (e.g. acute fasting, chronic sub-nutrition, postnatal under- or overfeeding, and high-fat diet) have an impact on the hypothalamic Kiss1 system, congruent with the associated change of pubertal timing	Rat, mouse (55, 56, 69, 70, 71)
Challenging data	
Congenital lack of <i>Kiss1</i> (e.g. <i>Kiss1</i> KO mice: mild delay) seems less deleterious for puberty than inactivation of <i>Gpr54</i>	Mouse (10 ^a)
Congenital ablation of Kiss1 neurons is compatible with pubertal maturation and fertility (developmental compensation?)	Mouse (21)
Open questions	
Is the role of kisspeptin signaling equally important in different mammalian (or non-mammalian) species?	
What are the differential roles of ARC vs RP3V Kiss1 neurons in the control of puberty in different species?	
What are the signals and mechanisms responsible for the developmental and activational program of Kiss1 neurons at puberty?	

^aNote that for the sake of concision, some of the references for the data above are embedded in extensive review articles quoted here.

Indeed, recent developments in the area have enlarged our knowledge on how kisspeptin release is putatively regulated and *Kiss1* neuronal activity controlled (23). Efforts on this front have allowed the identification of neurotransmitters that are coexpressed with kisspeptins at specific neuronal populations. As a paradigmatic example, expression of neurokinin B (NKB) has been demonstrated in a subset of *Kiss1* neurons in numerous species, including the sheep (24), the goat, the mouse (25, 26), the rat (27), and the monkey (28). The translational relevance of such coexpression, and of NKB actions in the reproductive brain, is reinforced by data from human studies that demonstrated that loss-of-function mutations in the genes encoding either NKB (*TAC3* in humans) or its receptor, NK3R (*TAC3R* in humans), are associated with impuberism and iHH (29, 30, 31), a phenotype similar to that of *GPR54* or *KISS1* null humans (10). Yet, NK3R null mice appear to display normal pubertal timing in males and females (32). To add further complexity, dynorphin-A (Dyn) and NK3R have also been found in *Kiss1*/NKB expressing neurons (25); *Kiss1*/NKB/Dyn colocalization being a specific feature of the ARC/infundibular population of *Kiss1* neurons, which has been renamed as KNDy to emphasize the potential usage of these three neuropeptides (23).

In keeping with the hypogonadotropic phenotype of patients with inactivating mutations of the NKB system, a number of studies in ewes, monkeys, mice, and rats have documented stimulatory actions of the NKB agonist, senktide, on LH secretion (28, 33, 34, 35). Of note, the stimulatory effects of senktide on LH secretion were abrogated in *Gpr54* null mice, thus suggesting that these require the integrity of kisspeptin signaling to manifest (35), a contention that is also supported by recent findings in the monkey (36). Notwithstanding these solid pharmacological data, evidence for null or even inhibitory responses to NKB agonists has also been presented (37, 38). The basis for these discrepant observations remains partially unknown, but they may stem from differences in the age, sex, species, and prevailing gonadotropin levels. Indeed, evidence for inhibitory effects of senktide on LH secretion comes mostly from animal models with pre-elevated LH concentrations, namely gonadectomy (GNX) without adequate sex steroid replacement (37, 38, 39). In this context, proof of desensitization of NKB-induced LH responses has been presented in the monkey (28). This desensitization might account for the null or even inhibitory effect of senktide on LH secretion in GNX rodents and ewes (25, 33, 34, 37), as they are expected to have pre-elevated, endogenous levels of NKB. Anyhow, the hypogonadotropic phenotype of humans with inactivating mutations of the NKB pathway, together with the reported effects of NKB agonists on LH release under physiological conditions in different species, solidly document the predominant stimulatory role of NKB in the central control of the HPG axis.

Integration of the above neuroanatomical, pharmacological, and genomic data has led to the proposal that KNDy neurons in the ARC/infundibular nucleus are key elements in the neuronal drive for the generation of GnRH pulses (12). In this interconnected network, kisspeptins would operate as a major output signal, responsible for the direct activation of GnRH neurons. In turn, NKB may act on KNDy neurons to finely tune (predominantly, stimulate) kisspeptin release, therefore inducing GnRH secretion in an indirect manner. This possibility is, at least partially, supported by a solid body of experimental evidence, including the following observations: i) central senktide injection induces expression of *C-fos* in ARC KNDy neurons (34); ii) senktide elicits LH secretion in a GnRH-dependent manner (28); iii) the LH-releasing effects of senktide are not detected in the absence of proper *Gpr54* signaling (35); iv) desensitization to the effects of continuous NKB stimulation takes place at a level upstream of GnRH neurons (28); v) substantial NK3R expression is detected in KNDy, but not GnRH neurons, in rodents and sheep (23); and vi) senktide induces the electrical activation of *Kiss1* neurons, as revealed by electrophysiological recordings in *Kiss1*-CreGFP mice (40). To add further refinement to the system, the third partner of the KNDy trio, Dyn, has been long recognized as an inhibitor of gonadotropin secretion, likely via its ability to repress the release of kisspeptins on GnRH neurons (12). Thus, the balance and reciprocal actions of NKB and Dyn would be a major determinant for the dynamic secretion of kisspeptins and hence for the generation of pulsatile GnRH and LH secretion.

Considering the relevant role of NKB signaling in the central control of the reproductive axis, the involvement of NKB in the onset of puberty has begun to be evaluated recently; yet, the number of studies on this particular area remains scarce (39, 41, 42). Notwithstanding, our data on immature rats demonstrate that, like adult, cyclic females, prepubertal female rats are able to respond to the NKB agonist, senktide, with robust LH responses (41). In addition, as is the case in adulthood, during puberty, expression of the genes encoding NKB and NK3R is found in hypothalamic areas, such as the ARC, with key roles in the central control of the gonadotropic axis. In fact, the hypothalamic expression of the mRNAs coding for NKB and NK3R increases during the infantile-to-juvenile transition, while blockade of NKB signaling by the use of a specific antagonist induces a modest but detectable delay in the timing of puberty (41). As a whole, the available evidence suggests that the NKB (auto)regulatory system of KNDy neurons, as described earlier for sexually mature animals, is operative and likely to play a role during pubertal maturation in rodents. Indeed, comparison of expression profiles of *NKB* and *Kiss1* genes in the hypothalamus of female rats during postnatal development would suggest that the rise of *NKB* expression anticipates the elevation of *Kiss1*

mRNA levels, a phenomenon that has been recently documented in the mouse (43), and whose functional implications are yet to be elucidated. In the same vein, a modulatory role of NKB signaling on puberty has been very recently proposed in the female sheep (42), in which increased NKB fiber immunoreactivity, but not cell numbers, was detected in post-pubertal vs pre-pubertal ewes. One interesting issue that warrants investigation is the potential sexual dimorphism in the roles of NKB in the control of puberty, at least in some species, as our recent data suggest that LH responses to senktide are lower in magnitude in male vs female rats before puberty, and, in contrast to females, they become null in male rats during the pubertal transition to adulthood (39). A synoptic compilation of the clinical and experimental data supporting a role of NKB pathways in the central control of puberty is presented in Table 2.

Metabolic control of puberty: leptin, kisspeptins, and beyond

Among its different modifiers, the amount of energy (fat) stores and the metabolic status of the organism are key regulators of puberty onset (44, 45). This is especially evident in the female, so that threshold energy reserves are needed to proceed through puberty as fertility, and specifically pregnancy and lactation are coupled to a marked metabolic drainage. Nonetheless, this phenomenon also takes place in the male and even in situations of sustained energy excess, such as obesity, which may also be linked to altered puberty (46). Considering the rising incidence worldwide of early-onset body weight disorders, ranging from anorexia to child obesity, and the worrisome observations of altered trends for the timing of puberty in various countries (47, 48), the analysis of the molecular and neurohormonal mechanisms for the joint control of metabolism,

energy homeostasis, and puberty onset has gained momentum, and exciting developments have taken place recently on this front.

As extensively revised elsewhere, clinical and experimental evidence accumulated during the last 15 years has documented the essential roles of the adipose hormone, leptin, whose levels in circulation are proportional to the size of body fat stores, in the metabolic control of puberty and fertility (44, 49). However, how and through which pathways leptin conducts such biological function are yet to be fully settled. Nonetheless, compelling evidence, coming from different species, including humans (46, 50), has demonstrated that, rather than a trigger, leptin operates as a permissive factor that allows puberty to proceed if sufficient body energy reserves are attained. Regarding the sites and mechanisms of action of leptin, the situation is less clear. On one hand, it is assumed that leptin is able to ultimately modulate the GnRH neuronal system (51). On the other hand, this action appears to be conducted indirectly, via intermediate afferents, as GnRH neurons are devoid of functional leptin receptors, as was anticipated by expression studies in rodents and primates (52, 53), and confirmed recently by functional genomic approaches, which demonstrated that selective elimination of leptin receptors eventually expressed in GnRH neurons did not overtly impair puberty or fertility (54). In addition, leptin failed to induce activation of STAT3, which is the primary effector of the hypothalamic actions of leptin effects at the hypothalamus, in GnRH neurons. Altogether, the available evidence strongly suggests that, under physiological conditions, the regulatory actions of leptin on GnRH secretion, and hence on puberty, are conducted via indirect mechanisms (54).

Recognition of such an indirect mode of action of leptin on GnRH neurons raised the question of what are the primary targets of leptin in the reproductive brain

Table 2 Synoptic compilation of the clinical and experimental data supporting a role of NKB pathways in the central control of puberty in various species. For further details, see section ‘Interactive partners of Kiss1: roles of neurokinin B in the control of puberty’.

	Species
Supporting evidence	
Genetic inactivation of NKB or its receptor (NK3R) results in hypogonadotropic hypogonadism and lack of puberty	Human (29, 30, 31)
Transient blockade (antagonism) of NKB signaling modestly delays puberty onset	Rat (41)
Hypothalamic expression of <i>Tac2</i> mRNA (encoding NKB) and NKB-positive fibers increase during prepubertal maturation	Rat, mouse, and sheep (41, 42, 43)
Conditions of metabolic stress that alter puberty (e.g. acute fasting and high-fat diet) have an impact on the hypothalamic NKB system, congruent with the associated change of pubertal timing	Rat (41, 71)
Activation of NKB signaling can (partially) rescue puberty onset in conditions of negative energy balance	Rat (41)
Challenging data	
Despite sub-fertility, <i>Nk3r</i> KO mice do not display gross pubertal alterations	Mouse (32)
Some forms of genetic inactivation of NKB signaling appear to be reversible	Human (31)
Open questions	
Is the role of NKB subordinated to or independent of that of kisspeptins in the control of puberty?	
What are the signals and mechanisms responsible for the control of NKB pathways before and during puberty?	
Are there important species differences (e.g. human vs rodents) in the roles of NKB in the control of puberty?	

and through which pathways are the effects of leptin conveyed. The initial recognition of the essential roles of kisspeptins as gatekeepers of puberty onset prompted analyses on the metabolic regulation of *Kiss1* neurons in general and of the specific effects of leptin on the hypothalamic *Kiss1* system. On the former, accumulating evidence has demonstrated that *Kiss1* neurons in the hypothalamus are sensitive to different forms of metabolic stress and thus may operate as conduits for the transmission of metabolic cues to the centers controlling puberty (18, 46). As an example, situations of negative energy balance, such as acute fasting, have been shown to suppress *Kiss1* mRNA expression and kisspeptin content in the hypothalamus of pubertal rats (55, 56). Conversely, administration of kisspeptin is sufficient to partially reverse the state of hypogonadotropism and delayed puberty caused by chronic under-nutrition in prepubertal female rats (55). Similar observations have been made in adult models of metabolic stress (57, 58, 59, 60), where the hypothalamic *Kiss1* tone is supposedly inhibited and the hypogonadotropic state rescued by exogenous kisspeptin.

In this context, recognition of the expression of the mRNA encoding the functional leptin receptor, *LepRb* or *Ob-Rb*, in ARC *Kiss1* neurons in the mouse and sheep (59, 61), and the demonstration of the ability of leptin, at high doses, to increase the hypothalamic expression of *Kiss1* gene in different models of severe metabolic stress, such as the leptin-deficient *ob/ob* mouse and the diabetic rat, fueled the hypothesis that leptin acts directly on *Kiss1* neurons to conduct its stimulatory/permissive effects on GnRH neurons (57, 58, 59). This possibility was further backed up by the demonstration of the capacity of leptin to enhance *Kiss1* mRNA levels in neuronal cell lines (58) and to induce the electrical firing of *Kiss1* neurons via activation of TRPC channels in the guinea pig (62).

Despite this suggestive evidence, recent data have challenged such a predominantly direct mode of action of leptin on *Kiss1* neurons. First, selective elimination of *LepR* in *Kiss1* cells appears to be compatible with grossly preserved puberty onset and fertility (63). It is noted, however, that the selectivity of *LepR* ablation in postnatal *Kiss1* neurons in the hypothalamus might be compromised by the fact that the *Kiss1* gene is widely expressed in other brain and other peripheral tissues at early stages of development. Moreover, congenital elimination of *LepR* in *Kiss1* cells might have caused compensatory changes that may obscure the impact of leptin signaling in this neuronal population. Nonetheless, an independent study mapping the distribution of the functional *LepRb* in various hypothalamic areas did not find evidence for its expression in GnRH or *Kiss1* neurons, except for a small population of *Kiss1* cells in the ARC (64). Of interest, an uncharacterized population of *LepRb*-expressing neurons has been identified in the ARC and RP3V, in close vicinity to *Kiss1* neurons (64). Thus, it is possible that a substantial part of the positive effects of leptin on the hypothalamic expression of

Kiss1/kisspeptin is conducted indirectly and transmitted via as yet unknown, intermediate circuits (64).

In the same vein, solid evidence has now demonstrated that the reproductive effects of leptin are, at least partially, carried out via primary actions outside the major *Kiss1*-expressing nuclei in the hypothalamus. A clear example is the ventral premammillary nucleus (PMV), which has recently emerged as an important primary target and transmitting node for the permissive effects of leptin onto GnRH neurons (63, 65). Neurons in the PMV express *LepRb* and respond to leptin stimulation, whereas ablation of this nucleus abrogated leptin effects on the reproductive axis. These observations would suggest the existence of *Kiss1*-independent pathways for the transmission of leptin effects onto GnRH neurons. Notwithstanding this, in face of the solid data suggesting the regulation of *Kiss1* expression by metabolic cues in general and leptin in particular, it remains possible that PMV and kisspeptin circuits may interplay or converge at some point for transmitting metabolic information to the GnRH system. A schematic presentation of the potential pathways transmitting leptin effects on GnRH neurons is shown in Fig. 1.

Additional aspects of the metabolic control of puberty, and the mechanisms and neurotransmitters involved, have also drawn interest and attention in recent years. For instance, the central molecular mediators for this phenomenon remain scarcely characterized. Efforts in this front have included the identification of the roles of

Figure 1 Schematic representation of the potential mechanisms for transmitting the actions of leptin, produced by the white adipose tissue (WAT) in proportion to body energy stores, to GnRH neurons in the basal forebrain, as a major output pathway for the downstream control of the gonadotropic axis and hence puberty onset. The putative modes of action may include: (1) direct actions of leptin on *Kiss1* neurons, which in turn may project to GnRH neurons; (2) indirect actions of leptin on neuronal afferents projecting on *Kiss1* neurons; and/or (3) leptin actions on hypothalamic (or extra-hypothalamic) nuclei devoid of *Kiss1* neurons. For the latter, the case of the ventral premammillary nucleus (PMV) is highlighted. For option 3, interplay with *Kiss1* circuits cannot be ruled out and is represented as dotted line projection. For further details, see section 'Metabolic control of puberty: leptin, kisspeptins, and beyond'. BBB, blood–brain barrier.

the cellular energy sensor, mammalian target of rapamycin (mTOR), in mediating the effects of leptin in the metabolic regulation of puberty. The physiological roles of mTOR in the control of cellular energy homeostasis and food intake have been extensively revised elsewhere (20). Experimental studies in pubertal female rats have suggested that, in addition, central mTOR signaling may play a relevant role in the control of puberty, as demonstrated by the fact that chronic blockade of mTOR by central injection of rapamycin markedly altered the onset of puberty, documented by delayed vaginal opening, reduced uterus and ovarian weights, and perturbed follicular maturation and anovulation (66). In good agreement, central inactivation of mTOR also counteracted the permissive/stimulatory effect of leptin on pubertal progression, so that the rescue of puberty onset induced by leptin injection to pubertal female rats subjected to under-nutrition was blocked by central coadministration of the mTOR inhibitor, rapamycin (66). From a mechanistic perspective, the impact of mTOR blockade on puberty onset and reproductive function is mediated, at least partially, by the inhibition of central kisspeptin circuits, as rapamycin was able to suppress *Kiss1* mRNA levels in key hypothalamic nuclei, such as the ARC (66). Altogether, the above observations suggest the existence of a leptin–mTOR–kisspeptin pathway that would participate in the metabolic regulation of puberty (20). Of note, the neuroanatomy of such a pathway is yet to be elucidated. In fact, recent studies revealed that the downstream target of mTOR, pS6, is not apparently expressed in *Kiss1* neurons (67), hence reinforcing the view of a predominant indirect mode of action of leptin/mTOR signals on kisspeptin pathways.

Additional areas of interest in the metabolic control of puberty are the identification of early influences and additional transmitters involved. On the former, the possibility that early forms of metabolic stress may have a durable influence in the timing of puberty is appealing given rising incidence of child obesity and the trends for advancement of the age of puberty in humans, a phenomenon that is likely detrimental for later (metabolic and cardiovascular) health and life expectancy (68). In this context, our studies in models of postnatal under- and over-nutrition in female rats have documented that rats with restricted feeding during lactation are leaner during the pubertal transition and displayed delayed vaginal opening at the time of puberty, despite they were allowed to eat *ad libitum* from weaning onward. In contrast, overfed female rats during lactation because of breeding in small litters showed earlier entry into puberty (69). In both extremes, there was a tight correlation between body weight, circulating leptin, and hypothalamic *Kiss1*/kisspeptin levels, so that leaner animals had low serum levels of leptin and reduced *Kiss1* expression and numbers of kisspeptin-positive neurons at the hypothalamus, while heavier rats had higher leptin concentrations and increased *Kiss1* mRNA levels and

kisspeptin-positive fibers in the RP3V immediately before puberty (69). Recent data have confirmed our previous (rat) observations in female mice submitted to postnatal under-nutrition (70). On the other hand, studies in models of metabolic stress in pubertal animals have recently revealed that the hypothalamic NKB system is also sensitive to conditions of negative energy balance, so that acute fasting not only suppressed *Kiss1* mRNA expression in the ARC and RP3V but also inhibited hypothalamic expression of the genes encoding NKB and NK3R in pubertal female rats (41). Importantly, the delay of puberty caused by under-nutrition was partially prevented by administration of an NKB agonist, thus supporting that NKB signaling may cooperate with kisspeptins in the metabolic control of puberty (41). As further support for the sensitivity of NKB pathways to metabolic regulation during puberty, it has been recently demonstrated that feeding on a high-fat diet to female rats from weaning caused precocious puberty onset and increased expression of the gene encoding NKB, as well as *Kiss1*, in the ARC (71).

Nesfatin-1: novel player in the metabolic control of puberty

As reviewed in previous sections, different studies have allowed identification of leptin, kisspeptins, and NKB as essential regulators of puberty. Yet, as highlighted elsewhere in this review, experimental work in different species has also defined the important contribution of other central transmitters and peripheral hormones, such as glutamate, GABA, and ghrelin (just to name but a few examples) in the physiological control of puberty onset (72, 73). A recent addition to the growing list of pubertal modulators is nesfatin-1, as revealed by recent data on pubertal female rats (74, 75). The major features of this neuropeptide as a putative regulator of the timing of puberty in the female are summarized below and synoptically presented in Table 3.

Nesfatin-1 is one of the peptide products of the gene *NUCB2*, with the ability to conduct anorectic effects acting at central (hypothalamic) levels (75). The interest in the metabolic actions of this molecule was reinforced by the demonstration of its expression in hypothalamic areas with key roles in food intake control, such as the ARC, the paraventricular nucleus (PVN), and the lateral hypothalamus (LHA), and the fact that anorectic effects of nesfatin-1 appear to be independent of leptin signaling (75). Considering that obesity is frequently linked to leptin resistance, identification of signals with preserved capacity to suppress feeding despite desensitization to the satiety effects of leptin holds considerable interest not only from a physiological but also from a pharmacological perspective. Taking into account the close association between the central regulatory mechanisms of energy balance and puberty, and the possibility of

Table 3 Synoptic compilation of the experimental data supporting a role of nesfatin-1 in the central control of puberty. Note that the experimental evidence summarized below has been obtained in rats. For further details, see section 'Nesfatin-1: novel player in the metabolic control of puberty' and references (74, 75).

Role of nesfatin-1 in puberty onset: supporting evidence

Nesfatin-1 is an anorectic neuropeptide that likely signals energy sufficiency; it probably operates in a leptin-independent manner
NUCB2/nesfatin-1 mRNA levels increase in the hypothalamus (LHA, PVN, and SON) at puberty
 Total nesfatin protein content in the hypothalamus increases at puberty
 Central injection of nesfatin-1 increases LH secretion in pubertal male and female rats
 Conditions of metabolic stress that delay puberty (e.g. acute fasting and chronic sub-nutrition) reduce the hypothalamic expression of *NUCB2*/nesfatin-1 at the peptide and mRNA levels
 Experimental suppression of hypothalamic levels of *NUCB2*/nesfatin-1 delays puberty onset
 Suppression of hypothalamic levels of *NUCB2*/nesfatin-1 moderately lower *Kiss1* expression

LHA, lateral hypothalamic area; PVN, paraventricular nucleus; SON, supraoptic nucleus.

leptin-independent pathways modulating puberty onset, we explored the possibility that nesfatin-1 might play a role in the central control of puberty.

Preclinical studies conducted by our group have supported this hypothesis. Hypothalamic *NUCB2*/nesfatin-1 expression increased during the pubertal transition, with a significant elevation of its mRNA levels in the LHA, PVN, and the supraoptic nucleus, and a threefold increase of its total protein content (74). In addition, conditions of negative energy balance known to perturb puberty, such as chronic sub-nutrition or acute fasting, decreased hypothalamic *NUCB2* mRNA and protein levels in pubertal females (74). All in all, these data suggest that during puberty, the hypothalamic expression of *NUCB2*/nesfatin-1 is subjected to precise developmental and metabolic regulation.

The relevance of nesfatin-1 signaling in puberty onset is further supported by functional analyses of the effects of its activation or inactivation. In this sense, central injection of low doses of nesfatin-1 evoked moderate but significant increases in serum LH levels in peripubertal female rats fed *ad libitum*. Intriguingly, gonadotropin responses to nesfatin-1 were substantially enhanced in conditions of short-term fasting, despite the significant decrease in the prevailing gonadotropin levels and of the hypothalamic expression of *NUCB2*/nesfatin-1 (74). Moreover, greater LH responses have been detected in pubertal male and female rats after infusion of higher doses nesfatin-1 (Navarro, Ruiz-Pino & Tena-Sempere, unpublished observations). In good agreement, suppression of the endogenous tone of nesfatin-1 in the hypothalamus during the pubertal transition, using an antisense morpholino oligonucleotide (as-MON) against *NUCB2*, impaired the normal timing of puberty, as evidenced by a delay in vaginal opening and the lowering of LH levels and ovarian weights. The

possibility that such reproductive changes might be due to a primary metabolic impact of the as-MON treatment can be ruled out as, despite the substantial decrease in the content of *NUCB2* protein in the hypothalamus, this treatment did not modify body weight gain or daily food intake during puberty (74).

Whether, in addition to its pubertal role, nesfatin-1 signaling is involved in the control of the gonadotropic axis in adulthood is yet to be defined. Our initial analyses did not find any gonadotropin responses to central injection of low doses of nesfatin-1 in adult female rats. In the same vein, i.c.v. infusion of as-MON against *NUCB2* did not affect the preovulatory surges of LH and FSH in adult, cyclic female rats (74). These observations point out a preferential action of nesfatin-1 in the control of the gonadotropic axis at puberty. Yet, recent preliminary data indicate that, at higher doses, nesfatin-1 can stimulate LH secretion in adult male rats (76) and mice (Navarro, Ruiz-Pino & Tena-Sempere, unpublished observations). This would suggest that the adult gonadotropic axis can also respond to the stimulatory effects of nesfatin-1, although sensitivity is likely lower in adult age. In any event, the features of nesfatin-1 summarized earlier qualify this novel factor as a putative metabolic regulator of puberty and gonadotropic function (75). In keeping with this view, our preliminary data suggest that blockade of endogenous nesfatin-1 tone, by the use of as-MON against *NUCB2*, induces a modest but significant decline in *Kiss1* gene expression in the ARC, whereas absence of Gpr54 signaling abrogates LH responses to nesfatin-1 (Navarro, Ruiz-Pino & Tena-Sempere, unpublished observations). While the neuroanatomy for such a putative nesfatin-*Kiss1* pathway is yet to be unraveled, these initial data suggest the convergence of nesfatin-1 and kisspeptin signaling in the central control of puberty and the gonadotropic axis.

Novel mechanisms in the control of puberty: roles of epigenetics and microRNAs

The intricate nature of puberty, as a complex developmental event sensitive to numerous regulatory cues, makes tenable that its timing does not solely rely on the transcriptional control of multiple pathways (as those summarized in previous sections) but may also depend on additional regulatory elements, such as epigenetics and microRNAs (miRNAs). While characterization of the physiological roles of these alternative/complementary mechanisms is still in its infancy, their potential involvement in mammalian puberty is appealing, as defined on the basis of preliminary data (see below) and conceptually challenging, as these mechanisms, which had not been linked to puberty until recently, might have escaped from conventional endocrine/molecular analyses.

Departing from the assumption that the timing of puberty in the general population is the consequence of the concerted interplay of sets of genes, effort has been devoted recently to identify new candidate genes/pathways in the control of puberty onset by means of genome-wide association studies (GWAS) (4). While one initial study reported an association with the SPOCK locus that has not been replicated (77), four independent papers published in May 2009 identified an association of the age at menarche (AAM) with variability at 6q21, in or near the *Lin28B* gene (78, 79, 80, 81). The robustness of this finding is reinforced by its recent confirmation in an extensive meta-analysis (82), and the fact that *Lin28B* had also been linked to breast development and adult height. *Lin28B*, and the related *Lin28A*, are RNA-binding proteins whose major known function is to block the processing of miRNAs of the *let7* family, via inhibition of the maturation of *let7* precursors (83, 84). Whereas some of the above GWAS detected other potential associations, analysis of the pubertal roles of *Lin28B* and related factors is especially appealing given the functional features of *Lin28B* (potential relation to AAM, breast development, and height) and its putative implication in the regulation of miRNAs. In good agreement, over-expression of the related *Lin28A* has been recently shown to delay puberty in mice (85), among other phenotypic features.

The above observations were the first to point out the possibility that miRNAs of the *let7* family (or eventually other miRNAs) may contribute to the control of puberty in humans and other mammalian species. The family of *let7* encompasses a group of closely related miRNAs, encoded by different clusters (four in humans), whose maturation is under the control of *Lin28B* and other related factors, such as *myc* (83, 84, 86, 87). In turn, *let7* miRNAs participate in the regulation of *Lin28B* expression, which is also putatively controlled by other miRNAs, such as *mir-132* and *mir-145* (as predicted by bioinformatic algorithms), and *myc* itself. Before the GWAS indicated earlier (78, 79, 80, 81), the potential roles of *let7* miRNAs and related factors in the control of puberty or other neuroendocrine functions had not been suspected. On the contrary, *let7* miRNAs were initially cataloged as putative tumor suppressors (88). Intriguingly, tumor-suppressor genes have been involved in the transcriptional control of puberty, with a detectable increase in its expression at the time of puberty (89). Whether *let7* miRNAs fit into this category remains to be elucidated.

Although the potential role of the *Lin28B/let7* tandem in the central control of puberty onset is yet to be defined, preliminary data from expression analyses strongly suggest that this is a tenable possibility. Thus, we have detected robust expression of *Lin28B*, as well as of the related *Lin28A*, mRNA in rodent hypothalamus, where their relative levels display a marked decline from the neonatal to the pubertal period (Sangiao-Alvarello,

Manfedi-Lozano & Tena-Sempere, submitted); this trend seems to be specific to the hypothalamus as it was not detected in the cerebral cortex. In addition, we have found expression of *let7a* and *let7b* in rat hypothalamus, with an inverse relationship with *Lin28B* mRNA levels; hence, a significant increase in relative levels of *let7* miRNAs is detected in the hypothalamus of male and female rats along postnatal/pubertal maturation. While these observations need to be extended, they emphasize the interest of specific expression and functional analyses of hypothalamic *let7* miRNAs and their binding proteins, *Lin28A* and *Lin28B*, as putative regulators of puberty.

In addition to miRNAs, preliminary evidence is mounting that the regulation of key components of the central pathways governing puberty onset may also involve epigenetic mechanisms. Conceptually, epigenetics, defined as the inheritable information that is not encoded by the mere nucleotide sequence of a given gene (90), is nicely suited for such a complex biological role, as epigenetic changes might participate in both long-term developmental modifications induced by gene–environment interactions, as well as in rapid changes of specific pathways, as those seen at the time of puberty (3). Indeed, epigenetics has been shown to participate in the control of several neurobiological functions (3); yet, little attention has been paid to date to its potential roles in mammalian puberty.

Among the different mechanisms for epigenetic control of gene expression, DNA methylation and histone modifications are among the best characterized and the most relevant (90, 91, 92). Incorporation of methyl groups to CpG islands is the only form of epigenetic modification of DNA, which is catalyzed by a family of DNA methyltransferases (DNMT). While DNMT1 is responsible for basal methylation, *de novo* methylation depends mostly on DNMT3A and DNMT3B. DNA methylation is a general mark of gene repression; the effectors of such an inhibitory effect on transcription are a family of methyl-CpG binding proteins (90, 91). In contrast, epigenetic changes on histones have been shown to involve a diversity of potential post-translational modifications, including acetylation, methylation, and phosphorylation (92). The acetylation status of histones is controlled by the concerted action of histone acetyl transferases and deacetylases (HDAC), acetylation being generally a mark of transcriptional activation (92). Histone methylation is brought about by different methyltransferases (HMT), the biological consequence being repression or activation depending on the histone and residue subjected to methylation (92). Furthermore, the final outcome in transcriptional regulation depends on the combination of epigenetic changes (e.g. DNA methylation plus histone acetylation and methylation), thus providing a higher degree of complexity to this regulatory system.

As stated in previous sections, puberty is the end point of a maturational continuum that begins during brain sex differentiation, which is driven by sex steroids acting during critical developmental windows (93). Evidence for a role of epigenetics in the control of sexual differentiation of specific nuclei has been presented recently. Thus, neonatal treatment with inhibitors of HDAC was capable to prevent the masculinization of the Bed Nucleus of the Stria Terminalis (94). The possibility of epigenetic control of brain sex differentiation is tremendously appealing, as changes in the epigenome of specific neuronal populations may contribute to the long-lasting, organizing effects involved in such a phenomenon. Yet, its functional consequences for the subsequent timing of puberty remain unknown. Nonetheless, our preliminary studies involving administration of inhibitors of either histone deacetylation or DNA methylation during the critical period of brain sex differentiation have disclosed a discernible impact of these epigenetic alterations on the timing of puberty (Leon, Castellano & Tena-Sempere, unpublished). Yet, the magnitude and ultimate mechanisms for such alterations, as well as the potential effects on other related systems (e.g. growth and energy homeostasis), await further characterization.

In addition to early developmental changes, epigenetic modifications may also contribute to the control of transient, dynamic changes in the neuroendocrine pathways governing puberty onset. This possibility has begun to be tested recently. Thus, pioneering studies by Ojeda *et al.* (3) have revealed that profound changes in the patterns of DNA methylation take place at the hypothalamus during puberty. Moreover, pharmacological blockade of DNA methylation or histone deacetylation in juvenile female rats has been shown to delay the timing of puberty (3). Roughly similar results have been obtained by our group in initial phenotypic and hormonal analyses of rat models of central pharmacological manipulation of methylation (e.g. treatment with 5'-AZA; inhibitor of DNMT) or deacetylation (e.g. treatment with valproic acid; blocker of HDAC) during the pubertal transition; yet the magnitude of some of the effects was modest (Leon, Castellano & Tena-Sempere, unpublished). Altogether, these studies strongly suggest that disturbance of epigenetic modifications of DNA/histones has an acute impact on the timing of puberty. Intriguingly, although the above manipulations (inhibition of DNA methylation or blockade of deacetylation) should preferentially result in gene activation, due to the removal of epigenetic marks associated with silencing, the final outcome observed in those experiments was a delay in the onset of puberty. Therefore, it has been hypothesized that, in physiological conditions, epigenetic changes would operate to inhibit the expression of puberty-repressor genes at the hypothalamus (3), whose identity and ultimate targets are yet to be fully elucidated. In this context, and considering its biological profile, one

tempting possibility is that the *Kiss1* gene is under epigenetic regulation. While evidence for this phenomenon has not been so far published in relation to puberty, recent observations strongly suggest that the differential methylation of the *Kiss1* gene might contribute to the sexual dimorphism in the expression levels of *Kiss1* in the RP3V (95), while estrogen-induced changes in histone acetylation at the *Kiss1* promoter in this nucleus may play a role in the positive feedback responsible for the generation of the preovulatory surge (96). These findings pave the way for specific analyses on the changes of epigenetic marks on the *Kiss1* gene in the ARC and RP3V during the pubertal transition and for the assessment of the functional consequences of various epigenetic manipulations on the pubertal expression of *Kiss1*.

Translational medicine and the control of puberty: preclinical models and species differences

As described in previous sections, the mechanisms governing puberty onset have been the subject of active investigation during recent years. For obvious ethical and experimental reasons, these analyses have been conducted mostly in suitable preclinical models, mainly wild-type and genetically modified rodents. This raises the question of the translational relevance of some of the findings made in such species, especially as it concerns puberty, for which, despite important commonalities, differences in terms of hormonal profiles and neuroendocrine mechanisms have also been identified between primate and non-primate species (1, 97, 98). For instance, the secretory profiles of gonadotropins during the infantile and juvenile periods of postnatal maturation differ between species, and only primates display a long-lasting quiescent period during infancy when gonadotropin levels remain persistently low (99). However, in spite of these particularities, there is also strong evidence for the existence of common neuroendocrine mechanisms for the control of puberty in both primate and rodent species, which justify the use of preclinical models for experimental studies on puberty. An illustrative example on this front is the *Kiss1* system, whose role in puberty onset has been mostly documented by studies in preclinical (mainly rodent) models but that, nonetheless, appears to also play an important role in the control of primate (and human) puberty. A brief account of the commonalities and specificities of *Kiss1* roles in the control of primate puberty is provided below, as a means to illustrate the strengths and potential limitations of preclinical studies published in this area.

As indicated earlier in this review, genetic data strongly suggest that kisspeptin signaling is indispensable for attaining complete pubertal maturation in humans. This role has been substantiated by compelling

expression and functional data obtained in primates that support the important contribution of *Kiss1* pathways in puberty. Thus, hypothalamic *Kiss1* mRNA expression has been shown to increase during pubertal maturation in monkeys (100), a period when there is a rise in the pulsatile release of kisspeptin-54 in the median eminence of this species, which closely correlates with the enhancement of GnRH pulse frequency during this age (101). Moreover, repeated injections of kisspeptin-10 to monkeys at the end of the juvenile phase evoked a pattern of GnRH discharges similar to that seen at puberty (102), whereas kisspeptin antagonism suppressed GnRH secretion in prepubertal and pubertal monkeys (103). Altogether, these data are in good agreement with similar observations in non-primate species, mainly rodents and sheep, and do support a major activational role of kisspeptin signaling in the control of primate puberty. Of note, recent analyses have initiated the study of the molecular basis of kisspeptin activation at puberty in humans, and a number of transcription factors with proven roles in mammalian puberty (as evidenced by a combination of previous rodent and primate studies), such as TTF1, CUX1, YY1, and EAP1, have been shown to bind to the human *KISS1* promoter and to regulate *KISS1* transcriptional activity (104).

In spite of the above commonalities, data from primate studies have also highlighted potential divergences with rodents and other preclinical species. For instance, while most rodent studies on the changes of *Kiss1* neurons during pubertal maturation have focused on the roles of the RP3V population, consistent increases in *Kiss1* gene expression have been documented in the medio-basal hypothalamus of monkeys during puberty, thus suggesting a key role of ARC *Kiss1* neurons in primate puberty (100). Likewise, the mechanisms governing the pubertal activation of *Kiss1* neurons might partially differ between primates and rodents. For instance, a recent study in monkeys suggested that *Kiss1* neurons are under the inhibitory control of GABA pathways before puberty and that their pubertal activation is caused, at least partially, by a decrease in the GABAergic tone (105). However, there is no evidence so far published, suggesting an inhibitory action of GABA on *Kiss1* neurons in rodents. In the same vein, mouse studies have suggested that the pubertal enlargement of *Kiss1* neuron population in the RP3V requires the drive of some degree of estrogenic input (106, 107); yet, it is possible that a similar phenomenon is not physiologically relevant in primates, where the activation of the gonadotropic axis during puberty can occur largely in a gonadal-independent manner (99, 108). In any event, while these data stress the need to exercise caution when directly extrapolating results from one species to another, the studies on the pubertal roles of kisspeptins summarized here clearly illustrate the validity and the power of mechanistic analyses in preclinical models, as a means to unveil the basis of complex neuroendocrine phenomena, such as puberty.

Concluding remarks

As a fascinating maturational period in the life span, the study of puberty has attracted the attention of scientists for centuries. We have to humbly recognize that our knowledge of the neurohormonal signals and mechanisms responsible for the control of puberty in general, and for its modulation by metabolic cues in particular, is still very incomplete. It must be acknowledged, though, that important developments have taken place on this front during the last years. The present review does not intend to provide a comprehensive overview of all those major findings, whose in-depth coverage clearly exceeds the limits and scope of this work, but rather to provide a flavor of the progress of the field, by presenting a few paradigmatic examples of novel signals and the mechanisms that have been recognized in recent years as putative regulators of puberty. Further analyses of these and related pathways are expected to shed further light into the intimacies of one of the most complex, and intriguing, developmental phenomena, whose alterations in humans are raising concerns in the scientific community and lay public worldwide.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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