Neuroendocrine and Molecular Mechanisms for the Metabolic Control of Puberty: Recent Developments

Manuel Tena-Sempere

Abstract Because reproduction is an energy-demanding function, acquisition of reproductive capacity at puberty, especially in the female, is metabolically gated, as a means of preventing fertility in conditions of energy insufficiency. On the other hand, obesity has been shown to also impact the timing of puberty and may be among the causes for the earlier trends of pubertal age reported in various countries, especially in girls but probably also in boys. The metabolic control of puberty is the result of the concerted action of different peripheral hormones and central transmitters that sense the metabolic state of the organism and transmit this information to the various elements of the reproductive brain, ultimately affecting GnRH neurons. Much has been learned in recent decades about the important roles of different neuropeptide pathways that are essential for the control of pulsatile GnRH secretion at puberty and its modulation by numerous physiological (and eventually pathological) signals, including metabolic cues. Remarkably, the essential roles of kisspeptins, the products of the Kiss1 gene that operate through the receptor, Gpr54 (also named Kiss1R), have been recognized in the last few years. In addition, the involvement of other central transmitters and molecular mediators, which may interplay with kisspeptins or operate in a kisspeptin-independent manner, has been unveiled recently by a combination of genetic, neuroanatomical, physiological and clinical studies. In this chapter, we will discuss some recent advances in our understanding of the neuroendocrine and molecular bases of the metabolic control of the onset of puberty. Special emphasis will be paid to summarizing the putative roles of the hypothalamic Kiss1 system in mediating the metabolic modulation of puberty, either via direct or (preferentially) indirect pathways, and to present some of our studies addressing the potential interplay of kisspeptins with other presumable metabolic regulators of puberty. In addition, recent progress in the identification of central molecular mediators, such as mTOR and AMPK, that are putatively involved in the metabolic gating of puberty will be reviewed here. We expect that

M. Tena-Sempere (✉)
Department of Cell Biology, Physiology and Immunology, University of Córdoba; CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

Instituto Maimónides de Investigaciones Biomédicas (IMIBIC), Hospital Universitario Reina Sofia, 14004 Córdoba, Spain
e-mail: fi1tesem@uco.es

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_9

such information will help to better understand the physiological basis of normal puberty and its eventual perturbations in conditions of metabolic stress, ranging from anorexia to morbid obesity.

Introduction: Metabolic Regulation of Puberty: Physiological and Pathophysiological Implications

Reproduction is an energy-demanding function that, despite being essential for the perpetuation of the species, is dispensable at the individual level. Hence, in extreme conditions that challenge energy homeostasis, other body functions are prioritized while the reproductive axis is suppressed, therefore causing alterations in puberty and/or fertility problems. In this context, it is well known that the onset of puberty is influenced by the magnitude of body energy reserves and different nutritional and metabolic factors (Fernandez-Fernandez et al. 2006; Navarro et al. 2007). Indeed, critical thresholds of fat reserves are required to attain complete pubertal development (Frisch and Revelle 1970), a phenomenon that is especially important in the female because of the substantial energy demands of pregnancy and lactation (Casanueva and Dieguez 1999). However, such a tight association likely occurs in both sexes, and different reports suggest that conditions of metabolic stress can also perturb puberty onset in males (Tena-Sempere 2008). Furthermore, conditions of sustained energy excess, such as morbid obesity, are frequently associated with reproductive alterations, through as yet poorly characterized patho-physiological mechanisms. All in all, a better understanding of the signals and pathways responsible for the metabolic regulation of puberty will help to define the basis for perturbations of puberty in humans, whose incidence seems to be increasing and may have a deleterious impact in health later in life (Lakshman et al. 2009).

Compelling evidence suggests that a substantial component of the mechanisms for the metabolic regulation of puberty takes place at central levels, ultimately by the modulation of the secretory activity of GnRH neurons in the hypothalamus (Elias and Purohit 2012; Navarro and Tena-Sempere 2012; Xu et al. 2012). The central mode of action of various metabolic regulators of puberty has been illustrated by functional genomics analyses, which showed that neuronal elimination of the receptors for insulin or leptin impaired puberty onset (Bruning et al. 2000; Quennell et al. 2009). Likewise, different studies have documented the capacity of leptin, insulin and ghrelin to modulate GnRH secretion in different ex vivo and in vitro settings (Cunningham et al. 1999; Tena-Sempere 2007; Pralong 2010). However, the emerging consensus is that the actions of most of the metabolic hormones influencing puberty are not conducted directly on GnRH neurons but rather take place through intermediary neuronal pathways.

Thus, GnRH neuron-specific knockout of the leptin receptor did not alter the timing of puberty in female mice, which displayed preserved fertility as adults

(Quennell et al. 2009). Similarly, it was recently documented that selective elimination of insulin receptors from GnRH neurons did not cause detectable alterations in the timing of puberty or adult reproductive function in male and female mice (Divall et al. 2010). Admittedly, results from congenital knockout mice should be interpreted with caution because of the possibility of developmental compensatory mechanisms. Yet, the above findings, together with data from expression and functional analyses, strongly suggest that leptin or insulin signaling directly in GnRH neurons is dispensable for pubertal onset, at least in rodents, therefore pointing out the existence of indirect pathways that would operate as conduits for the transmission of key metabolic information to GnRH neurons.

Endocrine Control of Puberty and Its Modulation by Metabolic Cues: Role of Leptin

Since the critical fat mass hypothesis was initially postulated in the 1970s by Frisch and co-workers (Frisch and Revelle 1970), the mechanisms whereby metabolic signals and body energy status influence puberty onset and later fertility have been analyzed thoroughly. Research in this area underwent a complete revolution with the identification, in 1994, and the subsequent characterization of the major biological actions, of the adipose hormone, leptin. Indeed, in the last two decades, leptin has been unanimously recognized not only as a key element in the hormonal control of body weight and energy homeostasis but also as an essential neuroendocrine integrator, linking the magnitude of body fat stores to different neuroendocrine axes, including the reproductive system. While detailed recapitulation of the biological features of leptin is clearly beyond the scope of this chapter, it is important to stress that, given that leptin is secreted in proportion to the amount of white adipose tissue and hence fuel reserves, it operates as a signal for the size of body fat stores to different body systems; thus it is indispensable for proper adjustment of the functioning of numerous physiological systems to changes in metabolic conditions.

Particularly in the context of the reproductive axis, leptin plays a key role in the metabolic control of puberty and fertility (Casanueva and Dieguez 1999; Ahima and Flier 2000; Tena-Sempere 2007). Thus, conditions of leptin insufficiency, as observed in humans with low or null leptin levels as well as in different rodent models (Fernandez-Fernandez et al. 2006; Tena-Sempere 2007), are often linked to a delay in or absence of puberty and perturbed fertility (Roa et al. 2010). Concerning the effects of leptin on puberty onset, there was an initial debate on whether leptin was a trigger or, instead, a permissive signal for puberty to proceed. The consensus derived from different observations in humans and model species indicated that, while threshold leptin levels are required to achieve normal pubertal development (and to retain reproductive function in adulthood), leptin alone is not sufficient per se to trigger complete puberty onset. This finding is compatible with a predominantly permissive function of leptin on puberty, which has been better

characterized in females. Of note, however, leptin deficiency, such as in ob/ob mice, has been linked to reproductive defects in males also (Elias and Purohit 2012), whereas leptin replacement can rescue low testosterone and gonadotropin secretion during short-term fasting in men (Chan et al. 2003). Therefore, it is tenable that leptin plays a key role in the control of puberty in both sexes, albeit with some differences in terms of relative importance vs. other metabolic signals. In any event, recent studies have raised some doubts about the actual roles of leptin in some aspects of the metabolic control of the gonadotropic axis, e.g., the rescue of the hypogonadotropic state following the termination of short-term food deprivation can apparently take place in the absence of detectable changes of circulating leptin levels in rodents and sheep (Szymanski et al. 2007; True et al. 2011). Nevertheless, to our knowledge, no study has challenged the consensus view that proper leptin levels are mandatory for puberty to proceed.

Neuroendocrine Control of Puberty and Its Metabolic Modulation: Role of Kisspeptins

Because of the predominant, if not exclusive, indirect mode of action of metabolic signals in the regulation of GnRH neurons, much attention have been devoted to elucidating the mechanisms whereby different hormones, including leptin, regulate GnRH neurosecretion. Admittedly, different factors are likely to operate via different routes and mechanisms. For the sake of concision, much of the discussion below will focus on the putative pathways whereby leptin, as the key factor for the integral control of energy balance and puberty, transmits its regulatory effects to the elements of the reproductive brain, and how neuronal pathways using kisspeptins as transmitters may participate in this function.

The identification of kisspeptins as gatekeepers of puberty and fertility, back in 2003, was a major breakthrough that has revolutionized our understanding of the neuroendocrine mechanisms that control the reproductive axis. A recapitulation of the major features of kisspeptins, the product of the *Kiss1* gene that operate via the surface receptor, Gpr54 (also termed Kiss1R), is clearly beyond the aims of this chapter; recent extensive reviews of this topic can be found elsewhere (Pinilla et al. 2012). In any event, as a means of introduction to the later contents of this review, some brief account of the major features of kisspeptins as major regulators of puberty and its modulation by metabolic signals is provided below.

The initial observation that patients or mice with null mutations in *Gpr54* or *Kiss1* genes do not undergo puberty already suggested a prominent, indispensable role of kisspeptin signaling in the control of puberty. This function has now been substantiated by a wealth of experimental data indicating that, during puberty, the hypothalamic Kiss1 system undergoes an extensive and complex activational program that seems to be essential for proper pubertal timing (Roa and Tena-Sempere 2010; Tena-Sempere 2010). Key aspects of such developmental activation

during pubertal maturation are (1) a rise in the hypothalamic expression of the *Kiss1* gene and kisspeptin content in key hypothalamic areas; (2) an increase in the sensitivity to the stimulatory effects of kisspeptin in terms of GnRH/LH responses; (3) an increase in the number of GnRH neurons expressing Gpr54, as well as in Gpr54 signaling efficiency; and (4) a rise in the number of kisspeptin neurons and of their projections/appositions to GnRH neurons (Sanchez-Garrido and Tena-Sempere 2013).

In good agreement with the above evidence, pharmacological analyses demonstrated that repeated administration of kisspeptins was sufficient to advance the occurrence of phenotypic or hormonal indices of puberty in rodents and monkeys, whereas blockade of Gpr54 by means of a specific kisspeptin antagonist caused an overt delay in puberty onset in female rats (Pineda et al. 2010). Admittedly, however, recent data have suggested that, under some circumstances, kisspeptin signaling might be dispensable for puberty onset in rodents. It must be stressed, however, that the latter experimental data involved the extensive (albeit probably not complete) congenital ablation of Kiss1 neurons, a condition that is likely to bring about important developmental compensation (Mayer and Boehm 2011). In fact, when similar ablation approaches were applied to early juvenile mice, puberty was overtly altered following selective elimination of Kiss1 cells, confirming that puberty does indeed require the activational contribution of preserved kisspeptin signaling to proceed normally.

Because of their paramount importance in the control of the reproductive axis in general, and of puberty in particular, the possibility that Kiss1 neurons may participate in the metabolic control of puberty has received quite some attention in recent years. Expression and functional data do suggest that Kiss1 neurons are influenced by metabolic cues, a contention that has been mainly documented in conditions of severe negative energy balance, such as acute fasting, chronic subnutrition, uncontrolled diabetes and inflammatory challenge (Castellano et al. 2005, 2006, 2010; Roa et al. 2009). Furthermore, in those conditions, replacement with pharmacological doses of kisspeptins was sufficient to rescue many of the reproductive deficits associated with energy insufficiency. Altogether, these data indirectly support the view that Kiss1 neurons are key sensors of the metabolic state of the organisms and play a key role in transmitting this information, ultimately, to GnRH neurons (Navarro and Tena-Sempere 2012). These observations also raised the obvious question of which metabolic signals are responsible for such control of Kiss 1 neurons; due to its essential roles in the metabolic control of reproduction, leptin was considered to be a tenable candidate (Sanchez-Garrido and Tena-Sempere 2013).

The possibility that leptin might operate as a regulator of Kiss1 neurons was initially supported by reports showing the expression of the mRNA encoding the functional leptin receptor in Kiss1 neurons of the arcuate nucleus (ARC) in the mouse and sheep (Smith et al. 2006; Backholer et al. 2010); however, only a subset of Kiss1 neurons, whose abundance varied from <10 % to ~40 depending on the studies, apparently expressed leptin receptors (LepR) in the mouse (Cravo et al. 2011). In addition, conditions of defective leptin levels are linked to blunted

Kiss1 mRNA expression, especially in the ARC (Smith et al. 2006; Quennell et al. 2011); reduced numbers of kisspeptin neurons in the rostral hypothalamic area have been also reported in situations of hypoleptinemia (Quennell et al. 2011). In turn, leptin administration caused an increase in hypothalamic *Kiss1* gene expression in rodent models of leptin deficiency (Castellano et al. 2006; Smith et al. 2006; Luque et al. 2007; Backholer et al. 2010). In line with these observations, leptin enhanced *Kiss1* mRNA expression in mouse and human neuronal cell lines (Luque et al. 2007; Morelli et al. 2008), and leptin injections in a sheep model of low leptin levels and central hypogonadism due to leanness significantly increased hypothalamic *Kiss1* mRNA expression (Backholer et al. 2010). Furthermore, it has recently been shown that leptin can directly activate ARC Kiss1 neurons in the guinea pig (Qiu et al. 2011). Altogether, these data are fully compatible with (and highly suggestive of) a direct mode of action of leptin in the control of Kiss1 neurons.

Notwithstanding the above evidence, recent data have questioned the hypothesis that direct actions of leptin on Kiss1 neurons are indispensable for its role as a metabolic regulator of puberty and fertility. Thus, congenital elimination of leptin receptors selectively from *Kiss1* cells apparently did not cause alterations in the timing of puberty or adult fertility (Donato et al. 2011). Caution should be exercised when interpreting these data, however, as congenital elimination of leptin receptors from Kiss1 cells might have caused compensatory changes that could mitigate the phenotypic impact of selective elimination of leptin signalling. Yet, additional studies in sheep and rodents did support the possibility of a predominantly indirect mode of action of leptin on Kiss1 neurons. These studies did not find evidence of the presence of functional leptin receptors in Kiss1 (or GnRH) neurons, except for a small population of Kiss1 cells in the ARC (Louis et al. 2011). Intriguingly, however, that work identified a previously unknown neuronal population expressing leptin receptor, located in the ARC and periventricular areas of the rostral hypothalamus (globally termed RP3V); they were found in close proximity to Kiss1 neurons and might contribute to conveying the biological effects of leptin to Kiss1 (and GnRH) neurons (Louis et al. 2011).

All in all, the latter results suggest that a significant fraction of the effects of leptin on the hypothalamic Kiss1 system is conducted indirectly and transmitted via as yet uncharacterized pathways (Louis et al. 2011). These may involve circuits originating from (or projecting to) the ventral pre-mammillary nucleus (PMV), as recent evidence has conclusively documented the indispensable role of PMV pathways in conveying the permissive effects of leptin on puberty onset (Donato et al. 2009, 2011). In fact, recent data have demonstrated alterations of the Kiss1 system following PMV lesions (Donato et al. 2013), suggesting that leptin-sensitive pathways originating from the PMV may impinge on Kiss1 circuits for conveying at least part of the effects of leptin in terms of metabolic gating of puberty. To add further complexity to this phenomenon, it has been recently suggested that leptin can carry out direct effects on Kiss1 neurons but only after pubertal maturation (Cravo et al. 2013); the physiological relevance of this phenomenon in the control of puberty and later fertility awaits further investigation.

Neuropeptide Partners of Kisspeptins and Metabolic Regulation of Puberty: The Role of Neurokinin B

In the last few years, it has been recognized that a group of Kiss1 neurons in the ARC co-express at least two other relevant transmitters involved in the control of the gonadotropic axis, namely, neurokinin B (NKB) and dynorphin (Dyn; Lehman et al. 2010; Pinilla et al. 2012). This subpopulation of ARC Kiss1 neurons has been termed KNDy to recognize such neuropeptide diversity (Lehman et al. 2010). It must be stressed, however, that the percentage of ARC Kiss1 neurons that co-express NKB and/or Dyn seems to vary depending on the species and sex (Hrabovszky et al. 2012); hence, Kiss1-only (and NKB-only) neurons, which are not KNDy neurons, likely exist in the ARC. Nonetheless, the importance of NKB and Dyn as central regulators of the reproductive axis is illustrated not only by their conserved co-expression with kisspeptins in discrete neuronal populations of the ARC in several species, including rodents and primates, but also by the fact that inactivating mutations of the genes encoding NKB (*TAC3*) or its receptor, NK3R (*TAC3R*), in humans are associated with a state of hypogonadotropic hypogonadism (Topaloglu et al. 2009; Gianetti et al. 2010; Young et al. 2010), similar to that caused by defects in kisspeptin signaling.

In addition, compelling pharmacological data support the roles of NKB and Dyn in the central regulation of gonadotropin secretion. Thus, stimulatory actions of the NKB agonist, senktide, on luteinizing hormone (LH) secretion have been reported in different species (Billings et al. 2010; Ramaswamy et al. 2010; Navarro et al. 2011; Garcia-Galiano et al. 2012). In turn, different studies have documented the effects of Dyn as an inhibitor of GnRH/gonadotropin secretion. These functional data, together with the neuroanatomical features of KNDy neurons, which are profusely interconnected by numerous collaterals, provided the grounds for the hypothesis that this neuronal population in the ARC is essential for the generation of GnRH pulses, due to its capacity to produce a very potent stimulatory output signal (kisspeptin) to GnRH neurons. The release of kisspeptins, in turn, would be subjected to the reciprocal control of NKB and Dyn, acting as predominantly stimulatory and inhibitory modulators, respectively (Navarro et al. 2009; Navarro and Tena-Sempere 2012). While this model has been widely accepted and explains the integration of kisspeptins, NKB and Dyn in the dynamic regulation of GnRH/gonadotropin secretion, important functional aspects of the KNDy neuronal network are yet to be fully elucidated, and species differences in the physiological relevance of these neurons in the control of GnRH neurons may exist.

Regarding the metabolic modulation of the reproductive axis, recent studies in models of metabolic challenge, conducted mostly in pubertal rodents, have documented that, just as previously demonstrated for *Kiss1*, the NKB system is also influenced by conditions of negative energy balance. Thus, complete food deprivation, as a means to induce a profound state of energy deficit, caused a concomitant suppression of *Kiss1* mRNA levels, in the ARC and RP3V, and of the hypothalamic expression of the genes encoding NKB and its receptor in

pubertal female rats (Navarro et al. 2012). Of note, in another model of metabolic stress, such as chronic under-nutrition during the pubertal transition, the delay of puberty caused by this state of negative energy balance was partially reversed by chronic injections of the NKB agonist, senktide. Altogether, these findings support the concept that NKB likely cooperates with kisspeptins in the metabolic control of puberty (Navarro et al. 2012). This possibility is fully compatible with the proposed KNDy paradigm, in which NKB would participate in the central regulation of GnRH secretion by increasing the release of kisspeptins (Navarro and Tena-Sempere 2012). Also in keeping with a putative role for NKB signaling in the metabolic regulation of puberty, it was recently shown that high fat diet administered to immature female rats from weaning onwards caused a significant advancement of puberty onset and enhanced the expression not only of *Kiss1* but also of the gene encoding NKB in the ARC (Feng Li et al. 2012). To our knowledge, the physiological roles of the inhibitory signal, Dyn, in the metabolic control of puberty and fertility remain largely unexplored.

Other Neuropeptide Pathways for the Metabolic Control of Puberty: The Case of Nesfatin-1

In addition to kisspeptins and NKB, (many) other central transmitters are involved in the joint control of energy homeostasis, metabolism and the reproductive axis. One prototypical example is nesfatin-1, one of the peptide products encoded by the gene Nucleobinding-2 (*Nucb2*). Nesfatin-1 has been shown to act as an anorectic signal in the hypothalamus (Oh et al. 2006; Garcia-Galiano et al. 2010a). In fact, due to its function as a satiety signal in various species and its expression in hypothalamic areas with key roles in the control of food intake, such as the ARC, the paraventricular nucleus (PVN) and the LHA, nesfatin-1 has been proposed to play a role in the regulation of energy homeostasis (Garcia-Galiano et al. 2010a). In addition, nesfatin-1 has been recently shown to participate in the control of female puberty in the rat and, therefore, might participate in the central networks responsible for the metabolic control of pubertal maturation.

The experimental evidence supporting such a role in puberty derives from expression and functional studies. Thus, hypothalamic NUCB2/nesfatin-1 expression has been shown to increase during pubertal maturation. In contrast, conditions of negative energy balance that suppress or delay puberty onset, e.g., chronic sub-nutrition or short-term fasting, are associated with decreased hypothalamic *NUCB2* mRNA and protein levels in pubertal females (Garcia-Galiano et al. 2010b). In addition, central injections of low (pmol) doses of nesfatin-1 have been reported to induce significant LH secretory responses in peripubertal female rats (Garcia-Galiano et al. 2010b), whereas suppression of the endogenous tone of nesfatin-1 in the hypothalamus during the pubertal transition, by means of

the central infusion of an antisense morfolino oligonucleotide (as-MON) against NUCB2, delayed the timing of puberty and lowered LH levels and ovarian weights.

Interestingly, studies in adult female rats did not detect gonadotropin responses to central injection of nesfatin-1 at doses that were effective in peripubertal rats; neither did infusion of as-MON against NUCB2 affect spontaneous pre-ovulatory surges of LH and FSH (Garcia-Galiano et al. 2010b). However, recent preliminary data indicate that, at higher doses, nesfatin-1 can stimulate LH secretion in adult male rats (Tadross et al. 2010), as well as in adult mice (Navarro et al., unpublished observations). Altogether, these features strongly suggest that nesfatin-1 might operate as a putative effector for the metabolic regulation of puberty and gonadotropic function (Garcia-Galiano et al. 2010a). In keeping with this view, preliminary data from my laboratory suggest that blockade of endogenous nesfatin-1 tone, by the use of as-MON against NUCB2, induces a suppression of *Kiss1* gene expression in the ARC and RP3V in pubertal female rats, whereas absence of kisspeptin signaling (i.e., in Kiss1r null mice) blocks LH responses to nesfatin-1 (Navarro et al., unpublished observations). The latter finding suggests a putative interplay between nesfatin-1 and kisspeptin pathways in the central control of the HPG axis, which may be relevant for its modulation by metabolic cues.

Molecular Mediators for the Metabolic Control of Puberty

Efforts have also been devoted recently to identifying putative molecular mediators that, acting at central levels, may participate in the metabolic control of puberty. Admittedly, progress in the field is still limited and is mostly restricted to the characterization of potential mediators of leptin actions. Just as illustrative examples, the cases of the energy sensors, mTOR and AMPK, will be briefly summarized below.

The mammalian target of rapamycin (mTOR) and the downstream elements of its signaling cascade are known to play a key role as a metabolic gauge of the cell, linking external (nutrient and hormonal) signals and basic cellular processes (Schmelzle and Hall 2000; Martin and Hall 2005; Wullschleger et al. 2006; Chiang and Abraham 2007; Tsang et al. 2007). In addition, mTOR signaling in the hypothalamus, and specifically the rapamycin-sensitive mTORC1 pathway, has been shown to participate in the control of energy homeostasis at the whole body level (Cota et al. 2006; Woods et al. 2008). Thus, leptin has been reported to modulate the mTOR pathway in the ARC, as an effector mechanism for its anorectic actions (Cota et al. 2006). Admittedly, the roles of hypothalamic mTOR signaling in the control of energy balance are more complex and involve nuclei other than the ARC (Villanueva et al. 2009), where mTOR mediates the metabolic effects of other key hormones, such as ghrelin (Martins et al. 2012). In fact, nutritional and metabolic signals seem to modulate the mTORC1 pathway within the medial basal hypothalamus in a nucleus- and cell-specific manner, with

different (if not opposite) responses depending on the prevailing metabolic status (Villanueva et al. 2009).

Besides its role in energy homeostasis and the transduction of metabolic effects of key factors, such as leptin, hypothalamic mTORC1 signaling seems to play an important role in the control of puberty onset and in transmitting the positive/permissive effects of leptin on the reproductive axis (Roa et al. 2009). Thus, sustained blockade of central mTOR signaling, by means of repeated intracerebral administration of rapamycin, delayed the timing of puberty in female rats, as monitored by vaginal opening, reduced ovarian and uterus weights, perturbed ovarian follicular development and suppressed ovulation. Moreover, central inhibition of mTOR was sufficient to totally block the permissive effects of leptin on puberty onset in female rats subjected to chronic subnutrition, as a model of low endogenous leptin levels. In fact, while leptin was able to rescue the ovulatory failure induced by subnutrition in pubertal females, this effect was completely prevented by simultaneous administration of the mTOR inhibitor, rapamycin, into the brain. These observations strongly suggest a positive role for mTOR in the central control of puberty, which seems to be indispensable for leptin effects on brain centers driving the onset of puberty. Indeed, activation of central mTOR signaling, by means of central administration of L-leucine, stimulated LH secretory responses and partially rescued the state of low gonadotropin levels caused by chronic subnutrition in pubertal female rats (Roa et al. 2009). The available evidence strongly suggests that the positive action of mTOR on the HPG axis stems, at least partially, from its capacity to activate Kiss1 neurons, since persistent inhibition of central mTOR signaling significantly reduced *Kiss1* mRNA expression levels in the ARC and, to a lesser extent, the RP3V. The molecular and neuroanatomical basis for this putative leptin-mTOR-Kiss1 pathway is yet to be fully clarified, and it remains possible that as yet unknown mediators might be involved in linking mTOR signaling and *Kiss1* expression.

Another brain fuel-sensing mechanism that might participate in the central control of the HPG axis is the one involving the AMP-activated protein kinase (AMPK), a member of the metabolite-sensing protein kinase family (Naimi et al. 2009; Canto and Auwerx 2009). AMPK detects changes in the AMP/ATP ratio and hence in the cellular metabolic state. In conditions of energy deficit, when ATP is consumed and excess AMP accumulates in the cell, AMPK becomes activated, thus causing the phosphorylation and inactivation of diverse ATP-consuming metabolic cascades. As described for the mTOR pathway, brain AMPK signaling may be a pivotal regulator of energy balance and food intake. Activation of AMPK stimulates appetite (Kahn et al. 2005; Cota et al. 2007), and leptin has been shown to suppress hypothalamic AMPK activity, whereas ghrelin stimulates it (Andersson et al. 2004). Interestingly, AMPK and mTOR are mutually regulated, e.g., AMPK inactivates mTOR in different cell systems (Inoki et al. 2003; Tsang et al. 2007), and hence these two metabolic cell sensors have been proposed to reciprocally cooperate in the central control of energy homeostasis (Cota et al. 2006).

Whether the above interaction applies also to the metabolic regulation of the HPG axis is an appealing possibility but yet to be experimentally proven. Nonetheless, indirect evidence would support this possibility, as AMPK activation has been reported to suppress GnRH secretion in murine GT1-7 cells in vitro and to perturb the estrous cyclicity, as a proxy marker of ovarian cyclic function, in female rats (Coyral-Castel et al. 2008; Wen et al. 2008). In good agreement, preliminary data from studies conducted in our group, targeting specifically the roles of central AMPK in puberty, strongly suggest that activation of central AMPK signaling delays puberty onset and partially inhibits hypothalamic *Kiss1* expression in the ARC (Roa et al., unpublished observations). Altogether, these observations are compatible with a predominant inhibitory role for AMPK pathways in the central control of reproduction, which is in line with its function as a sensor of energy insufficiency and functional antagonist of mTOR.

Concluding Remarks

Puberty, a major life-changing developmental event with a major impact on growth, reproduction and metabolic health later in life, has been the subject of active clinical and experimental investigation over the last decades. Numerous studies have helped to unveil some of the neuro-hormonal and molecular bases of the close connection between metabolism and body energy stores on the one hand and pubertal maturation on the other. Indeed, in recent years, we have learned much about the reciprocal metabolic and pubertal actions of several peripheral hormones and central transmitters, and we have begun to elucidate the pathways whereby these reciprocal connections take place. In this chapter, we aimed to provide a brief recapitulation of recent developments in the field, with special attention to the identification and functional characterization of novel neuropeptides and molecular effectors for the metabolic regulation of puberty. While this review does not include all of the major recent findings in this area, we believe that the information summarized here will provide a flavor of some of the most active research lines in this area and will endow the reader with a deeper understanding of the central mechanisms that link puberty to the metabolic state of the organism, not only in health but also in disease conditions ranging from anorexia to early-onset morbid obesity, whose prevalence is steadily increasing worldwide.

References

Ahima RS, Flier JS (2000) Leptin. Annu Rev Physiol 62:413–437

Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, Carling D, Small CJ (2004) AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem 279:12005–12008

Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ (2010) Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 151:2233–2243

Billings HJ, Connors JM, Altman SN, Hileman SM, Holaskova I, Lehman MN, McManus CJ, Nestor CC, Jacobs BH, Goodman RL (2010) Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. Endocrinology 151:3836–3846

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR (2000) Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125

Canto C, Auwerx J (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 20:98–105

Casanueva FF, Dieguez C (1999) Neuroendocrine regulation and actions of leptin. Front Neuroendocrinol 20:317–363

Castellano JM, Navarro VM, Fernandez-Fernandez R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M (2005) Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology 146:3917–3925

Castellano JM, Navarro VM, Fernandez-Fernandez R, Roa J, Vigo E, Pineda R, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M (2006) Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes 55:2602–2610

Castellano JM, Bentsen AH, Romero M, Pineda R, Ruiz-Pino F, Garcia-Galiano D, Sanchez-Garrido MA, Pinilla L, Mikkelsen JD, Tena-Sempere M (2010) Acute inflammation reduces kisspeptin immunoreactivity at the arcuate nucleus and decreases responsiveness to kisspeptin independently of its anorectic effects. Am J Physiol Endocrinol Metab 299:E54–E61

Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS (2003) The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest 111:1409–1421

Chiang GG, Abraham RT (2007) Targeting the mTOR signaling network in cancer. Trends Mol Med 13:433–442

Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, Seeley RJ (2006) Hypothalamic mTOR signaling regulates food intake. Science 312:927–930

Cota D, Proulx K, Seeley RJ (2007) The role of CNS fuel sensing in energy and glucose regulation. Gastroenterology 132:2158–2168

Coyral-Castel S, Tosca L, Ferreira G, Jeanpierre E, Rame C, Lomet D, Caraty A, Monget P, Chabrolle C, Dupont J (2008) The effect of AMP-activated kinase activation on gonadotrophin-releasing hormone secretion in GT1-7 cells and its potential role in hypothalamic regulation of the oestrous cyclicity in rats. J Neuroendocrinol 20:335–346

Cravo RM, Margatho LO, Osborne-Lawrence S, Donato J Jr, Atkin S, Bookout AL, Rovinsky S, Frazao R, Lee CE, Gautron L, Zigman JM, Elias CF (2011) Characterization of Kiss1 neurons using transgenic mouse models. Neuroscience 173:37–56

Cravo RM, Frazao R, Perello M, Osborne-Lawrence S, Williams KW, Zigman JM, Vianna C, Elias CF (2013) Leptin signaling in kiss1 neurons arises after pubertal development. PLoS One 8:e58698

Cunningham MJ, Clifton DK, Steiner RA (1999) Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod 60:216–222

Divall SA, Williams TR, Carver SE, Koch L, Bruning JC, Kahn CR, Wondisford F, Radovick S, Wolfe A (2010) Divergent roles of growth factors in the GnRH regulation of puberty in mice. J Clin Invest 120:2900–2909

Donato J Jr, Silva RJ, Sita LV, Lee S, Lee C, Lacchini S, Bittencourt JC, Franci CR, Canteras NS, Elias CF (2009) The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. J Neurosci 29:5240–5250

Donato J Jr, Cravo RM, Frazao R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr, Coppari R, Zigman JM, Elmquist JK, Elias CF (2011) Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J Clin Invest 121:355–368

Donato J Jr, Lee C, Ratra D, Franci CR, Canteras NS, Elias CF (2013) Lesions of the ventral premammillary nucleus disrupt the dynamic changes in Kiss1 and GnRH expression characteristic of the proestrus-estrus transition. Neuroscience 241:67–79

Elias CF, Purohit D (2012) Leptin signaling and circuits in puberty and fertility. Cell Mol Life Sci 70:841–862

Feng Li X, Lin YS, Kinsey-Jones JS, O'Byrne KT (2012) High-fat diet increases LH pulse frequency and Kisspeptin-Neurokinin B expression in puberty-advanced female rats. Endocrinology 153:4422–4431

Fernandez-Fernandez R, Martini AC, Navarro VM, Castellano JM, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M (2006) Novel signals for the integration of energy balance and reproduction. Mol Cell Endocrinol 254–255:127–132

Frisch E, Revelle R (1970) Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 169:397–399

Garcia-Galiano D, Navarro VM, Gaytan F, Tena-Sempere M (2010a) Expanding roles of NUCB2/nesfatin-1 in neuroendocrine regulation. J Mol Endocrinol 45:281–290

Garcia-Galiano D, Navarro VM, Roa J, Ruiz-Pino F, Sanchez-Garrido MA, Pineda R, Castellano JM, Romero M, Aguilar E, Gaytan F, Dieguez C, Pinilla L, Tena-Sempere M (2010b) The anorexigenic neuropeptide, nesfatin-1, is indispensable for normal puberty onset in the female rat. J Neurosci 30:7783–7792

Garcia-Galiano D, van Ingen Schenau D, Leon S, Krajnc-Franken MA, Manfredi-Lozano M, Romero-Ruiz A, Navarro VM, Gaytan F, van Noort PI, Pinilla L, Blomenrohr M, Tena-Sempere M (2012) Kisspeptin signaling is indispensable for neurokinin B, but not glutamate, stimulation of gonadotropin secretion in mice. Endocrinology 153:316–328

Gianetti E et al (2010) TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab 95:2857–2867

Hrabovszky E, Sipos MT, Molnar CS, Ciofi P, Borsay BA, Gergely P, Herczeg L, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z (2012) Low degree of overlap between kisspeptin, neurokinin B, and dynorphin immunoreactivities in the infundibular nucleus of young male human subjects challenges the KNDy neuron concept. Endocrinology 153:4978–4989

Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115:577–590

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1:15–25

Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, Wareham NJ, Ong KK (2009) Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab 94:4953–4960

Lehman MN, Coolen LM, Goodman RL (2010) Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 151:3479–3489

Louis GW, Greenwald-Yarnell M, Phillips R, Coolen LM, Lehman MN, Myers MG Jr (2011) Molecular mapping of the neural pathways linking leptin to the neuroendocrine reproductive axis. Endocrinology 152:2302–2310

Luque RM, Kineman RD, Tena-Sempere M (2007) Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. Endocrinology 148:4601–4611

Martin DE, Hall MN (2005) The expanding TOR signaling network. Curr Opin Cell Biol 17:158–166

Martins L, Fernandez-Mallo D, Novelle MG, Vazquez MJ, Tena-Sempere M, Nogueiras R, Lopez M, Dieguez C (2012) Hypothalamic mTOR signaling mediates the orexigenic action of ghrelin. PLoS One 7:e46923

Mayer C, Boehm U (2011) Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci 14:704–710

Morelli A, Marini M, Mancina R, Luconi M, Vignozzi L, Fibbi B, Filippi S, Pezzatini A, Forti G, Vannelli GB, Maggi M (2008) Sex steroids and leptin regulate the "first Kiss" (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. J Sex Med 5:1097–1113

Naimi M, Arous C, Van Obberghen E (2009) Energetic cell sensors: a key to metabolic homeostasis. Trends Endocrinol Metab 21:75–82

Navarro VM, Tena-Sempere M (2012) Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. Nat Rev Endocrinol 8:40–53

Navarro VM, Castellano JM, Garcia-Galiano D, Tena-Sempere M (2007) Neuroendocrine factors in the initiation of puberty: the emergent role of kisspeptin. Rev Endocr Metab Disord 8:11–20

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA (2009) Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci 29:11859–11866

Navarro VM, Castellano JM, McConkey SM, Pineda R, Ruiz-Pino F, Pinilla L, Clifton DK, Tena-Sempere M, Steiner RA (2011) Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. Am J Physiol Endocrinol Metab 300:E202–E210

Navarro VM, Ruiz-Pino F, Sanchez-Garrido MA, Garcia-Galiano D, Hobbs SJ, Manfredi-Lozano M, Leon S, Sangiao-Alvarello S, Castellano JM, Clifton DK, Pinilla L, Steiner RA, Tena-Sempere M (2012) Role of neurokinin B in the control of female puberty and its modulation by metabolic status. J Neurosci 32:2388–2397

Oh IS, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M (2006) Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443:709–712

Pineda R, Garcia-Galiano D, Roseweir A, Romero M, Sanchez-Garrido MA, Ruiz-Pino F, Morgan K, Pinilla L, Millar RP, Tena-Sempere M (2010) Critical roles of kisspeptins in female puberty and preovulatory gonadotropin surges as revealed by a novel antagonist. Endocrinology 151:722–730

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92:1235–1316

Pralong FP (2010) Insulin and NPY pathways and the control of GnRH function and puberty onset. Mol Cell Endocrinol 324:82–86

Qiu J, Fang Y, Bosch MA, Ronnekleiv OK, Kelly MJ (2011) Guinea pig kisspeptin neurons are depolarized by leptin via activation of TRPC channels. Endocrinology 152:1503–1514

Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Herbison AE, Grattan DR, Anderson GM (2009) Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology 150:2805–2812

Quennell JH, Howell CS, Roa J, Augustine RA, Grattan DR, Anderson GM (2011) Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology 152:1541–1550

Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM (2010) Neurokinin B stimulates GnRH release in the male monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology 151:4494–4503

Roa J, Tena-Sempere M (2010) Energy balance and puberty onset: emerging role of central mTOR signaling. Trends Endocrinol Metab 21:519–528

Roa J, Garcia-Galiano D, Varela L, Sanchez-Garrido MA, Pineda R, Castellano JM, Ruiz-Pino F, Romero M, Aguilar E, Lopez M, Gaytan F, Dieguez C, Pinilla L, Tena-Sempere M (2009) The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology 150:5016–5026

Roa J, Garcia-Galiano D, Castellano JM, Gaytan F, Pinilla L, Tena-Sempere M (2010) Metabolic control of puberty onset: new players, new mechanisms. Mol Cell Endocrinol 324:87–94

Sanchez-Garrido MA, Tena-Sempere M (2013) Metabolic control of puberty: roles of leptin and kisspeptins. Horm Behav 64:187–194

Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. Cell 103:253–262

Smith JT, Acohido BV, Clifton DK, Steiner RA (2006) KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol 18:298–303

Szymanski LA, Schneider JE, Friedman MI, Ji H, Kurose Y, Blache D, Rao A, Dunshea FR, Clarke IJ (2007) Changes in insulin, glucose and ketone bodies, but not leptin or body fat content precede restoration of luteinising hormone secretion in ewes. J Neuroendocrinol 19:449–460

Tadross JA, Patterson M, Wynne KJ, Patel S, Suzuki K, Ghatei MA, Bloom SR (2010) Nesfatin suppresses feeding and stimulates the hypothalamo-pituitary-gonadal axis. In: 14th international congress of endocrinology, p S442. Kyoto: Endocrine J.

Tena-Sempere M (2007) Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology 86:229–241

Tena-Sempere M (2008) Ghrelin as a pleotrophic modulator of gonadal function and reproduction. Nat Clin Pract Endocrinol Metab 4:666–674

Tena-Sempere M (2010) Roles of Kisspeptins in the control of hypothalamic-gonadotropic function: focus on sexual differentiation and puberty onset. Endocr Dev 17:52–62

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358

True C, Kirigiti MA, Kievit P, Grove KL, Susan Smith M (2011) Leptin is not the critical signal for kisspeptin or luteinising hormone restoration during exit from negative energy balance. J Neuroendocrinol 23:1099–1112

Tsang CK, Qi H, Liu LF, Zheng XF (2007) Targeting mammalian target of rapamycin (mTOR) for health and diseases. Drug Discov Today 12:112–124

Villanueva EC, Munzberg H, Cota D, Leshan RL, Kopp K, Ishida-Takahashi R, Jones JC, Fingar DC, Seeley RJ, Myers MG Jr (2009) Complex regulation of mammalian target of rapamycin complex 1 in the basomedial hypothalamus by leptin and nutritional status. Endocrinology 150:4541–4551

Wen JP, Lv WS, Yang J, Nie AF, Cheng XB, Yang Y, Ge Y, Li XY, Ning G (2008) Globular adiponectin inhibits GnRH secretion from GT1-7 hypothalamic GnRH neurons by induction of hyperpolarization of membrane potential. Biochem Biophys Res Commun 371:756–761

Woods SC, Seeley RJ, Cota D (2008) Regulation of food intake through hypothalamic signaling networks involving mTOR. Annu Rev Nutr 28:295–311

Wullschleger S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. Cell 124:471–484

Xu Y, Faulkner LD, Hill JW (2012) Cross-talk between metabolism and reproduction: the role of POMC and SF1 neurons. Front Endocrinol (Lausanne) 2:98

Young J, Bouligand J, Francou B, Raffin-Sanson ML, Gaillez S, Jeanpierre M, Grynberg M, Kamenicky P, Chanson P, Brailly-Tabard S, Guiochon-Mantel A (2010) TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 95:2287–2295