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Kisspeptins and Puberty

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Abstract

Puberty is a complex developmental phenomenon, driven by brain pathways under the modulation of external and internal cues, which culminates with the acquisition of reproductive competence and sexual maturity. From a neurobiological perspective, this process is incumbent to the timed activation of the population of hypothalamic neurons producing gonadotropin-releasing hormone (GnRH); the enhancement of GnRH neurosecretory activity being ultimately responsible for the triggering of puberty. In the last decade, kisspeptins have emerged as pivotal upstream regulators of GnRH neurons, with prominent roles in their activation during the pubertal transition and its (direct or indirect) modulation by different regulatory signals, including metabolic cues. We will briefly review herein the major features of kisspeptins and their producing neurons, the so-called *Kiss1* neurons, as major gatekeepers and fine regulators of reproductive maturation and puberty onset in mammals.

Introduction

Puberty is major developmental event in the lifespan of any individual, which culminates with the attainment of sexual (somatic, psychological) maturity and reproductive capacity[1]. This intricate maturational phenomenon is grounded on early differentiation events (starting in utero), and involves a complex series of morphological, functional, endocrine, behavioral and psychological changes, which ultimately lead to the acquisition of a complete adult phenotype[1]. Accordingly, puberty is regarded not only as a specific, relatively narrow stage of development, but rather considered as the final output of a maturational continuum that leads to reproductive competence.

The tempo of puberty is dictated by the dynamic interplay between genetic and environmental factors[1], so that perturbation of such dialogue often results in the inappropriate development of the reproductive axis that commonly leads to alterations of the timing of puberty (precocious, delayed or absent). In this sense, beyond its paramount biological relevance, puberty may be considered as putative sentinel for perturbations of the gene-environment interactions along early stages of development, whose alterations may lead to deregulation of key homeostatic systems. In fact, changes in the timing of puberty, especially earlier puberty, might impact important development events, including somatic and psychological maturation, and appear to be linked to numerous adverse health outcomes[2], as well as reduced life expectancy[3]. This is specially worrying given the reported trends for changes in the age of puberty (mostly earlier), which seem to be more frequent in girls but appear to occur also in boys[4-6]. These observations urge for a better understanding of the physiological control of puberty, and of the pathophysiological basis of its alterations.

Neuroendocrinology of puberty: the GnRH Secretrory drive

The neuroendocrine system responsible for puberty onset is the so-called hypothalamic-pituitary-gonadal (HPG) axis, whose full activation permits the acquisition of reproductive competence at puberty[7, 8]. This neurohormonal system primarily in-

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tegrates three major groups of factors, which are: (a) the decapeptide, GnRH, which is released in a pulsatile manner to the portal circuit connecting the medial-basal hypothalamus and the pituitary; (b) the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), produced by gonadotropic cells of the anterior pituitary; and (c) the gonadal hormones, mainly sex steroids and several peptides, which operate via feedback loops at upper levels of the HPG axis to conduct its homeostatic regulation. In addition, the HPG axis is also modulated by other endogenous factors, e.g., metabolic hormones, and exogenous signals, e.g., nutrition and light conditions[8], which contribute also to the dynamic control of puberty.

From its reproductive perspective, the initiation of puberty is founded on the heightening of the pulsatile release of hypothalamic GnRH[9]. Yet, the intimate mechanisms whereby this increase occurs remain ill defined. The current view, articulated in the so-called "central drive" hypothesis, concurs that the increase in the pulsatile GnRH secretion leading to the onset of puberty is the result of changes in the activity of the central pathways controlling GnRH neurons, with a switch in the balance between excitatory inputs (which increase) and inhibitory signals (which decrease at the time of puberty), thus causing the *drive* for the pubertal activation of the HPG axis[9]. Notably, the activator afferents of GnRH neurons include not only transsynaptic inputs but also glial-born factors[10].

Kisspeptins and the central control of puberty

While the control of GnRH neurons is multifactorial, and numerous neuropeptides and transmitters have been shown in the last decades to regulate GnRH secretion, kisspeptins have emerged in recent years as fundamental regulatory signals, with an essential role in the control of puberty[11]. Kisspeptins are a family of structurally related peptides, encoded by the *Kiss1* gene, which act via the G protein-coupled receptor, Gpr54, also termed Kiss1R or kisspeptin receptor[11,12]. The reproductive facet of kisspeptins, and their role in the precise control of puberty, was surfaced by seminal observations, back in 2003, that inactivating mutations of GPR54 were linked to absence of puberty and hypogonadism of central origin (aka, hypogonadotropic hypogonadism) in humans[13, 14]. Such reproductive role was further demonstrated by the observation of similar phenotypes in patients with inactivating mutations of *KISS1* and in null mice for Gpr54 or *Kiss1*[11].

These findings boosted enormous interest and prompted the analysis of the physiological roles of *Kiss1* neurons in the regulation of GnRH neurosecretion and puberty onset. While detailed recapitulation of the results of these studies clearly exceeds the scope of this review, it is important to stress that such analyses have unambiguously documented a sophisticated developmental program of *Kiss1* neurons, responsible for their activation during the pubertal maturation[11,15]. This complex and multifaceted phenomenon includes, at least, the following major components: (a) an increase in the hypothalamic *Kiss1* mRNA/kisspeptin content during the juvenile-pubertal transition that drives the full activation of the GnRH/gonadotropin system[16-19]; (b) a rise in the sensitivity to the excitatory actions of kisspeptins on GnRH secretion[20,21]; (c) an enhancement of Gpr54 signaling efficiency in GnRH neurons; (d) a state of partial resistance to desensitization to kisspeptin stimulation[21, 22]; and (e) a rise of kisspeptin-positive neurons and their projections to GnRH neurons[23,24]. In good agreement, pharmacological blockade of kisspeptin signaling has been reported to delay the onset of puberty in female rats[25], whereas ablation of *Kiss1* neurons in the juvenile period prevented pubertal maturation in female mice[26].

Despite the solid clinical and experimental evidence suggesting a role of kisspeptins in the timing of puberty, one study using functional genomics to congenitally ablate *Kiss1* neurons suggested that kisspeptin signaling seems to be dispensable for the attainment of female fertility[26]. In the same vein, another study in a mouse model causing a congenital 95% reduction in hypothalamic *Kiss1* expression was compatible with roughly preserved reproductive function in male mice[27]; yet, a similar reduction in hypothalamic *Kiss1* levels severely altered fertility in female mice. A tenable explanation is that these phenotypes might be due to incomplete neuronal/Kiss1 expression elimination, coupled to potential developmental compensation[26], which can be activated by such congenital manipulations. Thus, these findings would not refute the essential role of hypothalamic kisspeptins in the physiological timing of puberty, but rather suggest that redundancy in *Kiss1* signaling may help to safeguard reproduction in relatively adverse conditions, at least in rodents.

Putative regulators of kisspeptins: neurokinin B and leptin

Two major populations of *Kiss1* neurons have been identified in the hypothalamus, as characterized in detail in rodents[28]. One is located in the rostral hypothalamus, mainly at the antero-ventral periventricular nucleus (AVPV), while the other is placed in the arcuate nucleus (ARC) or its equivalent infundibular region in humans[28,29]. The latter, which is primarily involved in the tonic control of pulsatile

gonadotropin secretion, have been shown to co-express the neuropeptides, neurokinin-B (NKB) and dynorphin (Dyn), which seemingly operate as co-regulators of kisspeptin neurons and eventual modulators of puberty onset[30,31]. Therefore, this neuronal population has been named KNDy neurons, as they express Kiss1, NKB and Dyn. Admittedly, the level of co-expression of these neuropeptides varies depending on the species, the sex and the state of the HPG axis[32,33]. In any event, compelling evidence suggest that NKB may operate as a major stimulatory signal for the auto-regulation of kisspeptin release by KNDy neurons, and hence an important component of the machinery responsible for pulsatile GnRH secretion. In turn, Dyn would conduct an opposite role, by inhibiting kisspeptin output from KNDy neurons, and thereby suppressing GnRH neurosecretion.

Suggestive, as yet incomplete evidence suggests a role of NKB in the control of puberty onset. Thus, it has been shown that central injection of the NKB agonist, senktide, in prepubertal female rats stimulates LH secretion[34], while the hypothalamic expression of NKB gene increases before the rise of *Kiss1* mRNA levels in female rodents[35]. In the same line, central blockade of NKB signaling caused a moderate delay of puberty onset in female rats [34]. Recent evidence has shown that the stimulation of NKB signaling or the inhibition of Dyn induces early puberty onset in female rats [36]. These findings are compatible with the view that the inhibitory effect of Dyn on kisspeptin neurosecretion decreases at the time of puberty, while the stimulatory effect of NKB gradually augments during this period. As a result of these sequential events, the pulsatile kisspeptin/GnRH/LH release would increase, thus leading to puberty onset[37].

Another group of putative modulators of kisspeptin signaling are the metabolic factors, which are known to be potent modifiers of puberty onset. Among them, a major player is the adipose hormone, leptin[38]. The unequivocal essential role of leptin in the control of puberty is attested by the alterations in pubertal timing observed in conditions of leptin deficiency or (morbid) leptin excess, which are often associated with delayed or advanced puberty, respectively[39,40]. In this context, experimental evidence has suggested that leptin is a positive modulator of *Kiss1* neurons; a phenomenon that can contribute to transmitting the metabolic modulation puberty onset. Thus, conditions of leptin deficiency are known to suppress hypothalamic *Kiss1* expression in rodents and sheep[39,40], while leptin administration activates kisspeptin neurons and/or increase *Kiss1* expression in different species[41-44], and cell lines[45,46]. In addition, expression of the gene encoding the leptin receptor has been documented in a fraction of ARC kisspeptin neurons in mice and ewes[42,47]. Nonetheless, whether leptin modulated *Kiss1* neurons directly or indirectly has been the subject of intense debate, and studies involving selective elimination of leptin receptors from *Kiss1* neurons congenitally, using the Cre-loxP technology, suggest that leptin signaling in this neuronal population is dispensable for attainment of reproductive competence[48], while other studied have failed to detect a significant amount of functional leptin receptors in *Kiss1* neurons before puberty[49,50]. These findings, together with the observations that leptin does influence *Kiss1* neurons, would suggest a predominantly indirect mode of action, in which intermediary pathways and signals would play a relevant role. Among these, neuronal pathways originating from the ventral pre-mammillary nucleus (PMV)[51], and others using nitric oxide as major transmitter[52].

Conclusions And Future Directions

Puberty, as fascinating developmental phenomenon, has drawn considerable attention, and numerous clinical and experimental studies, conducted in different mammalian species during the last decades, have contributed to substantially expand our understanding of the physiology of puberty and the basis of some of its major alterations. In this context, the discovery of kisspeptins, as essential regulatory elements for the central control of puberty in mammals, including humans, has revolutionized our knowledge about the intimate mechanisms of puberty, allowing to surface novel regulatory pathways whereby different factors, from environmental cues to endogenous hormones, contribute to the precise timing of puberty. While the progress has been astonishing, important aspects of kisspeptin and pubertal physiology remain ill defined and will likely focus considerable attention in the coming years. Among these facets, we foresee that elucidation of the molecular mechanisms for the precise control of Kiss1/kisspeptin expression and the relative role of the different *Kiss1* neurons populations will be of special interest, as epitomized by recent exciting findings on the putative roles of epigenetic regulatory mechanisms and microRNAs in the central control of puberty [53-55].

Disclosure

No potential conflict of interest in relation to this presentation.

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