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HYPOTHALAMO-PITUITARY AXIS AND PUBERTY

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ABSTRACT

Puberty is a complex process that culminates in the acquisition of psychophysical maturity and reproductive capacity. This elaborate and fascinating process marks the end of childhood. Behind it lies a complex, genetically mediated neuroendocrine mechanism through which the gonads are activated thanks to the fine balance between central inhibitory and stimulating neuromodulators and hormones with both central and peripheral action. The onset of puberty involves the reactivation of the hypothalamic-pituitary-gonadal (HPG) axis, supported by the initial "kiss" between kisspeptin and the hypothalamic neurons that secrete GnRH (the GnRH "pulse generator"). This pulsatile production of GnRH is followed by a rise in LH and, consequently, in gonadal steroids.

The onset of puberty varies naturally between individuals, and especially between males and females, in the latter of whom it is typically earlier. However, pathological variations, namely precocious and delayed puberty, are also possible. This article reviews the scientific literature on the physiological mechanisms of puberty and the main pathophysiological aspects of its onset.

KEYWORDS

GnRH pulse generator; kisspeptin; KNDy neuronal network; endocrine disrupters; precocious puberty; delayed puberty

1. INTRODUCTION

The term “puberty” refers to a set of psycho-neuro-endocrine changes that occur between the end of childhood and the achievement of final height and complete sexual maturation, resulting in sexual dimorphism and the production of gametes. This process is specific to species that procreate by sexual reproduction. The normal duration of puberty is about 5-6 years. The onset of puberty is significantly later in males than in females. In females, the first signs are normally seen at between 8.5 years and 12.5 years (on average 10.5 years), compared with between 9.5 and 13.5 years (on average 11.5 years) in males. Several factors can influence the timing of puberty onset, giving rise to precocious puberty or delayed puberty (Marshall & Tanner, 1969 and 1970).

All the clinical modifications that occur during puberty are the direct consequence of hypothalamic-pituitary-gonadal axis (HPG) activation, involving an increase in gonadotropin-releasing hormone (GnRH) pulsatility and, consequently, in follicle-stimulating hormone (FSH), luteinizing hormone (LH) and gonadal steroids. The purpose of this paper is to carry out a review of the literature regarding the physiological mechanisms of the HPG axis that lead to pubertal activation and the factors that influence the timing of puberty. It is also described a detailed analysis of the main pathophysiological and clinical aspects of puberty.

2. WHEN DOES THE ACTIVITY OF THE HPG AXIS BEGIN?

The HPG axis has three activation periods: during foetal life, with a peak between mid-gestation and subsequent decrease towards the end of pregnancy; after birth, between the first week of life and six-nine months, the period classically known as mini-puberty; and after childhood, with the onset of puberty (Guimiot et al., 2012). During embryogenesis, and in particular at around 40 days of gestation, the neurons that secrete GnRH originate from the epithelium of the medial olfactory

pit and migrate along nerve fibres to the foetal hypothalamus (Schwanzel-Fukada et al., 1989; Crossin et al., 1996).

The pituitary hormones FSH and LH, known as gonadotropins, begin to be detectable in the anterior pituitary and general circulation at 9 weeks of gestation (Kaplan et al., 1976; Clements et al., 1976). Hypothalamic input is probably required to maintain this secretion and to exert trophic effects on the pituitary gonadotropes. The regulation of foetal GnRH neuron activity also includes kisspeptin and KISS1R but the secretion of gonadotropins only becomes GnRH-dependent from 30-31 weeks of gestation (Guimiot et al., 2012).

Changes in LH and FSH concentrations during pregnancy are crucial for the activation of peripheral hormones involved in the maturation of the ovaries and testicles. Males and females have different gonadotropin concentrations throughout gestation. The first major difference is that females have a higher proportion of FSH than LH compared to males (Beck-Peccoz et al., 1991). During the first half of pregnancy, female foetuses have a greater concentration of both LH and FSH than males. This is probably due to the greater negative feedback exerted by testicular hormones in male foetuses (Clements et al., 1976; Kaplan et al., 1976; Reyes et al., 1973), or to a different sex steroid receptors expression between male and female foetuses. During the first trimester of pregnancy, placental hCG (peak around 8-12 weeks) plays an essential role in determining an increase in gonadal production of testosterone in male foetuses, thanks to its LH-like effect. This placental hCG-dependent testosterone increase allows the male differentiation of the foetus (i.e. masculinization of genitalia), showing that gonadotropins do not play a decisive role in sexual differentiation during the first trimester of pregnancy. However, in those rare cases of congenital absence of LH (LHB subunit), ambiguous genitalia do not occur. Instead, this condition is present in those rare forms of the receptor (LHCGR) or steroidogenic enzymes mutations.

Subsequently, in mid-pregnancy, female foetuses show a higher peak of FSH and LH levels than males, comparable to that seen in the menopause or in women with hypergonadotropic hypogonadism. Peak levels are accompanied by the first maturation of the ovarian follicles, in females, and the seminiferous tubules, in males. Their concentrations then drop gradually and are practically suppressed at birth (Massa et al., 1992). However, the maturation of ovarian follicles in female foetuses does not seem to be linked uniquely and directly to a pituitary stimulation, as a follicular production in foetuses with anencephaly, a condition in which gonadotropins are not produced given the absence of the pituitary gland, has been also described (Baker et al., 1980; Bizzarri & Cappa, 2020).

In the final period of gestation, there is a considerable increase in placental secretion of oestrogens to determine a suppression of the foetal HPG axis, and therefore a decrease of both gonadotropins (Kuijper et al., 2013). Immediately after birth, the high concentration of placental hormones endures, and continue to exert a negative feedback action on the hypothalamic GnRH. After a few days it begins to drop, unlocking the secretion of GnRH itself. Consequently, gonadotropin levels begin to increase about a week to 10 days after birth (Bergadá et al., 2006; Schmidt et al., 2000), giving rise to the so-called mini-puberty. As in gestation, the secretion of gonadotropins and gonadal steroids also differs between males and females during mini-puberty. Males, unlike females, have higher concentrations of LH than FSH, peaking between the second and tenth week of life, but also have an earlier reduction in these hormones, which usually return to suppression values at around 4-6 months (Kuiri-Hänninen et al., 2011). The trend of testosterone in males exactly reflects that of LH.

In females, gonadotropins peak between the first and the third month of postnatal life, similarly to males, but remain measurable for up to 3-4 years (Winter et al., 1975; Kuiri-Hänninen et al., 2011). Oestradiol levels show greater fluctuations than testosterone, and begin to drop at around the

sixth month of life (Schmidt et al., 2002; Chellakooty et al., 2003; Bidlingmaier et al., 1987). All these hormonal variations in both males and females are essential for postnatal gonadal maturation.

After mini-puberty, the HPG axis remains quiescent throughout childhood in both males and females, through a mechanism which is still unknown. It then reactivates upon the onset of puberty.

3. ONSET OF PUBERTY: THE GnRH PULSE GENERATOR, THE KNDy SYSTEM, EXCITATORY AND INHIBITORY NEUROMODULATORS

The increased frequency and amplitude of GnRH release from the hypothalamus to the hypophyseal portal system triggers the anterior pituitary secretion, thereby promoting the onset of pulsatile LH secretion.

The hypothalamic neural substrate that generates the pulsatile GnRH release is known as the "GnRH pulse generator", a term first coined in the early 80s (Pohl & Knobil, 1982).

In 2003, two independent groups reported that a mutation causing loss of GPR54 receptor (otherwise known as KISS1R) function resulted in hypogonadotropic hypogonadism in both males and females (De Roux et al., 2003; Seminara et al., 2003). The KISS1R ligand, better known as kisspeptin, is a peptide of 54 amino acids, first discovered as a tumour suppressor (Ohtaki et al., 2001). Other studies demonstrated that KISS1 gene inactivation results in the failure of pubertal progression (Topaloglu et al., 2012), further stressing the importance of this gene in pubertal development. In 1997, De Roux et al. described a case of familiar idiopathic hypogonadotropic hypogonadism related to compound heterozygous mutation of the GnRH-receptor gene (GNRHR), who presented only a partial progression of pubertal development (De Roux et al., 1997). Twelve years later, another gene, whose homozygous mutation was responsible for idiopathic

hypogonadotropic hypogonadism, was identified. Specifically, this gene encoded gonadotropin-releasing hormone 1 (GNRH1), and its mutation was found in two teenage siblings whose parents were heterozygous, and therefore not affected (Bouligand et al., 2009).

Studies on female rodents allowed the localization of two main groups of kisspeptin neurons in the hypothalamus. The largest is located in the arcuate nucleus (ARC), while the other is clustered in the preoptic area of the third ventricle (POA) in non-rodents (including humans), and in the anteroventral periventricular nucleus (AVPV) in rodents (Lehman et al., 2013). These two different populations of neurons could play different roles in the activation of the HPG axis (Pinilla et al., 2012): ARC kisspeptin neurons seem to have a negative feedback control on gonadotropin release, while POA neurons have a positive feedback.

Kisspeptin is a highly powerful GnRH secretagogue released from the ARC through a complex neuronal network that also involves other neuromodulators, some stimulating its secretion, and others inhibiting it. The stimulatory neuromodulators include neurokinin B (NKB), encoded by the tachykinin 3 (TAC3) gene (Herbison, 2015). In 2009, Topaloglu et al. found that mutations involving loss of function of the genes encoding the TAC3 gene (coding NKB) or the TACR3 gene (coding NKB receptor) were responsible for the failure of pubertal development and the onset of hypogonadotropic hypogonadism (Topaloglu et al., 2009). As a result, NKB was thought to stimulate the release of GnRH through kiss1 neurons.

Goodman et al. demonstrated that kisspeptin/NKB neurons in the ARC of sheep co-express a third peptide called dynorphin (Dyn). This endogenous opioid peptide, encoded by the prodynorphin gene, inhibits the secretion of kisspeptin (Goodman et al., 2007). This was confirmed by a later study demonstrating that the administration of a dynorphin receptor antagonist resulted in early puberty onset (Nakahara et al., 2013).

Given the interconnection between the two neuromodulators NKB and Dyn and kisspeptin, in 2010 Cheng et al. coined the acronym KNDy to precisely describe the set of neurons involved in the onset of puberty (Cheng et al., 2010). So, to summarise, the pulsatile secretion of GnRH is based on the coordinated activity of the KNDy neuronal network (Cheng et al., 2010), in which NKB stimulates the release of kisspeptin, while Dyn exerts an inhibitory action (Wakabayashi et al., 2010).

Other kisspeptin inhibitors and stimulants can be found upstream of the KNDy system. One of the key stimulants is glutamate, the main excitatory neurotransmitter in the brain, while GABA has an inhibitory effect (Clarkson et al., 2006; Watanabe et al., 2014). Studies conducted on monkeys confirmed the opposite role of these two neurotransmitters, showing that GABA levels gradually decrease before puberty, while glutamate levels are high at the beginning of puberty, before GnRH secretion (Shamas et al., 2015; Brann et al., 2002; Kurian et al., 2012). In particular, Kurian and co-authors demonstrated that an infusion of a GABAa receptor antagonist (bicuculline) within the stalk-median eminence of pre-pubertal monkeys could cause an increased release of kisspeptin, and consequently of GnRH secretion. The Authors also showed that the same stimulatory effect was lacking in the case of contextual infusion of the kisspeptin receptor antagonist (peptide 234).

As for GABA, neuropeptide Y also has an inhibitory effect on the system, whose levels tend to decrease with increasing GnRH secretion (Livadas & Chrousos, 2016).

RFRP, a neuropeptide in the RF amide family, is also a kisspeptin inhibitor. The human homologue is the RFRP-3 variant, the administration of which induces a clear reduction in LH secretion (Khan et al., 2011).

Recent studies showed that mutations involving heterozygous loss of function of the makorin ring finger 3 (MKRN3) gene, involved in protein ubiquitination and cell signalling, leads to central

precocious puberty (Abreu et al., 2013; Christoforidis et al., 2017; Grandone et al., 2017). MKRN3 is a maternal imprinted gene located on chromosome 15q11-q13, the same region involved in Prader-Willi syndrome (PWS), in which only the paternal allele is expressed (Jong et al., 1999). Specifically, loss of function mutations of the paternal allele lead to familial forms of central precocious puberty (Abreu et al., 2015). It is unknown how MKRN3 blocks puberty, however, it was found to be highly expressed before puberty within the medial basal hypothalamus of the arcuate nucleus of mice, which is the same brain areas that contain neurons secreting kisspeptin and NKB. On the contrary, its expression decreases as puberty advances (Abreu et al., 2015). Another paper by the same research group confirmed the presence of the expression of MKRN3 also in rats and monkeys, and its decrease during the prepubertal period, independently of gonadal activation (Abreu et al., 2020). They also demonstrated that MKRN3 represses transcriptional activity of the KISS1 (encoding kisspeptin) and TAC3 gene (encoding NKB), through an association with the respective promoters. Another recent study found the microRNA “mir-30” as a probable repressor of MKRN3, whose increase of expression with the approach of puberty induces the reduction of the MKRN3 activity and the consequent unblock of puberty (Heras et al., 2019).

All these observations obtained from animal studies along with the evidence of MKRN3 mutations in humans with central precocious puberty, support the hypothesis of a strong inhibitory role of this protein on the pubertal onset. However, further studies are needed to better understand this mechanism.

Finally, sex steroid hormones exert inhibitory effects on both GnRH release and kisspeptin neurons. Studies on rodents showed that neuroestradiol acts in a dual mode, having an inhibitory effect in the ARC and a stimulatory effect in the AVPV nucleus (Khan & Kauffman, 2012; Kauffman, 2010).

The onset of puberty also seems to be linked to metabolic and energy signals. Among the metabolic cues leptin, an adipocyte-derived hormone, plays a key role in regulating appetite and food intake and is essential for GnRH pulsatility via the KISS1 system (Sanchez-Garrido & Tena-Sempere, 2013; Zhang et al., 1994). In fact, states of nutritional deprivation and/or leptin deficiency reduce hypothalamic KISS1 expression and delay or prevent puberty (Castellano & Tena-Sempere, 2016). Another important metabolic hormone is ghrelin, a peptide secreted predominantly by the stomach, whose concentrations increase during fasting. Ghrelin cooperates with leptin in the control of puberty, but inhibits GnRH and LH release; the exact mechanisms underlying this regulation are still unknown (Sanchez-Garrido & Tena-Sempere, 2013; Tena-Sempere, 2013; Zhang et al., 1994).

A new neuroendocrine factor, involved in regulating food intake and therefore implicated in the release of gonadotropins and consequent onset of puberty, is known as pituitary adenylate cyclase-activating polypeptide (PACAP). The role of this polypeptide is not entirely clear yet, as it seems to exert both stimulator and inhibitor effects to the release of gonadotropins in rodents (Szabó et al., 2002). Since PACAP was found in the ventromedial hypothalamus nuclei involved in metabolic and reproductive functions, it could represent a direct or indirect leptin mediator, and be involved in its metabolic role (Tanida et al., 2013). Some studies showed that PACAP is also highly expressed at the ventral pre-mammillary nucleus. In particular, Ross and co-authors demonstrated that a high percentage of leptin-responsive neurons of the ventral pre-mammillary nucleus express PACAP, thus highlighting a central role in the direct modulation of neurons secreting kisspeptin. Its pivotal role on the pubertal onset is confirmed by studies on female mice, which showed that the PACAP removal from this population of neurons leads to a delay in the vaginal opening and first oestrus (Ross et al., 2018).

Table 1 provides a systematic list of all above-mentioned molecular mediators, with their respective inhibitory or excitatory effect on HPG axis.

4. MECHANISM OF ACTION OF GnRH AND GONADOTROPINS

Once the HPG axis is activated, the GnRH released from the hypothalamus binds a G-protein coupled receptor (GnRH-R receptor, 60 KDa) on the surface of the pituitary gonadotropin-secreting cells. The ligand binding and the consequent heterotrimeric G proteins activation triggers a mechanism that leads to the arousal of a phosphatidylinositol-calcium second messenger, which results in the synthesis and episodic secretion of both FSH and LH (Flanagan & Manilall et al., 2017).

The pulsatile release of GnRH alters the secretion patterns of pituitary gonadotropic cells. An increased frequency promotes the secretion of LH, while a lower frequency favours the release of FSH (Savoy-Moore & Swartz, 1987; Wildt et al., 1981). Continuous exposure to GnRH leads to downregulation of GnRH receptors and a decrease in gonadotropin synthesis and secretion (Rispoli & Nett, 2005). Gonadotropin synthesis is controlled by the transcription of the distinct β-subunits. FSH and LH contain a common α-subunit, but it is FSHβ and LHβ that produce the specific actions of gonadotropins (Childs et al., 1990). Like FSH and LH secretion, the transcription of the gonadotropin subunits is dependent on GnRH pulse frequency (Dalkin et al., 1989; Haisenleder et al., 1991; Kaiser et al., 1997).

In males FSH binds to receptors on the surface of Sertoli cells, activating proteins that regulate gene expression and the production of Sertoli cell proteins. These play important roles in supporting and regulating spermatogenesis within the seminiferous tubules (Rannikko et al., 1996). LH acts on Leydig cells to stimulate testosterone production, the main sex steroid hormone in males (Veldhuis et al., 1987).

In females, LH is essential for ovulation and the sustenance of corpus luteum function. It also contributes to follicular function and plays a key role in androgen production. FSH regulates oestradiol production in the granulosa cells and is the main promoter of follicular growth; in more advanced phases of follicular development, it synergizes with LH. (Richards & Pangas, 2010).

5. EFFECT OF ENDOCRINE DISRUPTOR CHEMICALS ON PUBERTAL ONSET

An endocrine disruptor chemical (EDC) is an exogenous substance or mixture that affects endocrine system function. Several works published in the early 2000s evaluated the effect of EDCs on the development of pubertal signs in girls and boys, such as age at menarche in girls, testicular volume increase in boys, and pubertal Tanner stages in both sexes. Most of these studies investigated girls, with few addressing male puberty. It is known that the EDCs interact with the organism through multiple mechanisms related to their hormone-like structural characteristics; among these, the direct interaction with the hormonal receptor or the post-receptor bio-synthetic pathways, the suppression of hormonal synthesis, and the direct toxic effects are the most significant. Regarding their influence on puberty, the androgenic, oestrogenic, anti-androgenic or anti-oestrogenic effects are crucial. Specifically, androgen-like and anti-oestrogenic effects are essentially related to the inhibition of aromatase activity; the oestrogen-like effect may occur through the stimulation of aromatase activity or direct binding to the oestrogen receptor; the anti-androgenic effect may depend on androgen receptor blockage or steroidogenic enzyme inhibition; finally, EDCs can have a direct effect on GnRH, resulting in an increased secretion of gonadotropins. Based on the prevailing activity of the ECD, this can result in an anticipated or delayed pubertal development.

In 2002, Den Hond et al. evaluated 80 boys with a mean age of 17.3 years who were exposed to Polychlorinated biphenyls (PCBs) and dioxin during the pubertal period. They found a negative

association between elevated serum PCB and pubertal stage, particularly genital maturation and pubic hair growth. There was also an association between higher PCB exposure and lower testicular volume. In contrast, dioxin had no effect on pubertal stage (Den Hond et al., 2002). Saiyed et al. and Guo et al. also reported some interesting results. Saiyed showed an association between pubertal exposure to endosulfan and a low level of pubic hair, testis and penis maturation (Saiyed et al., 2003), while Guo found reduced penile length in 55 boys living in the Chinese city of Yucheng who had experienced prenatal exposure to polychlorinated biphenyls and polychlorinated dibenzofurans, in comparison with 55 healthy subjects (Guo et al., 2004).

Many cross-sectional and longitudinal human studies have evaluated the association between the onset of puberty and prenatal or pubertal exposure to various potential endocrine disruptors. A 2008 study of 15 girls and 18 boys exposed to dioxin through contaminated breast milk, demonstrated delayed breast development in the girls and delayed age at first ejaculation for the boys (Leijs et al., 2008).

More recent reports include a study by Ferguson et al., who longitudinally analysed the prenatal or infantile effects of phthalates and bisphenol-A on 118 boys (aged 8–14). Prenatal exposure was negatively associated with the onset of the adrenarche and pubarche (with high SHBG levels), whilst infantile exposure also caused low testosterone levels, and hence a delay in the main signs of puberty (Ferguson et al., 2014).

A similar recent study evaluated the impact of in utero phthalate and bisphenol-A exposure on sexual maturation in 109 peripubertal boys aged 8–14 years. In the first and second trimesters, in utero exposure to DEHP was linked to increased peripubertal serum oestradiol levels. In the third trimester, exposure was associated with a delay in the onset of pubarche, with increased SHBG levels (Watkins et al., 2017).

A longitudinal study conducted on 516 boys considered the effects of organochlorine chemicals, lead (Pb) and non-dioxin-like-PCBs. The authors evaluated EDC concentrations at the age of 8-9 years, and subsequently carried out annual examinations until the age of 18-19. Pubertal stage and testis volume were evaluated during each clinical investigation. The main finding was that the persistence of Eds in blood negatively influenced growth during puberty; organochlorines and lead (Pb) delayed the onset of puberty, while PCBs tended to advance the timing of puberty (Sergeyev et al., 2017).

There are few original papers on the effects of EDCs on puberty in males, as the majority focus on female puberty. These may be because female puberty is more easily detectable, as the menarche is an undisputed sign of sexual maturation. However, the main findings from studies that do investigate the effects of EDC exposure on male puberty indicate that it is delayed. This is probably due to the oestrogenic-like effects of PCBs, PCDFs, and endosulfan. In any case, research in boys is more focused on semen quality, which is much more closely related to fertility. Table 2 summarises the main EDCs' characteristics and reports studies on animals or humans about their putative role on the HPG axis dysfunction.

6. EVOLUTION OF PUBERTAL STAGES, EARLY AND DELAYED PUBERTY

The onset and progression of puberty are clinically evaluated by the Tanner and Marshall stages in both males and females, to document the sequence of secondary sexual character modifications (Marshall & Tanner, 1969, 1970). In males, testicular growth, mainly due to the proliferation of seminiferous tubules, is often the first sign of puberty. This is indicated when a volume greater than 3-4 mL is reached together with the growth of the scrotum; this is followed by the appearance of pubic hair and enlargement of the penis, first in length and then in breadth. The first conscious ejaculation occurs at an average age of 13.5 years and normozoospermia is

obtained at around a bone age of 17 years (Laron et al., 1980). Other changes that occur during puberty include vocal timbre, pubertal growth spurt and the appearance of axillary and facial hair.

In a paper published in 2005 by our research group, the evolution of the male gonad during the different pubertal stages (from G1 to G5) was assessed in detail. Changes in gonadotropin, inhibin B (INHB) and testosterone concentrations were evaluated in relation to Tanner's pubertal stages and testicular volume. We found a direct correlation between INHB and FSH concentrations during pubertal stage G1 and, more generally, during the initial stages of testicular maturation. We also demonstrated a strong biphasic relationship between INHB and FSH during pubertal development, with inversion of this relationship in the mid-late stages of puberty: at this stage, (G3-G4) INHB increases as its inverse relationship with FSH is being established, leading to spermatogenesis. In addition, we found a statistically significant direct correlation between INHB and testicular volume in all pubertal stages (Radicioni et al., 2005).

In females, puberty culminates with the menarche, signalling the end of childhood and the beginning of reproductive capacity. The average age at menarche is 12.4 years (Lacroix & Langaker, 2019). However, the first sign of puberty in girls is breast development, that begins under the control of oestrogen at a mean age of 10 years (Klein et al., 2017). Pubic and axillary hair growth is controlled by adrenal androgens. The uterus and ovarian volumes increase, the vagina becomes longer, and the vaginal mucosa thickens and changes colour. The labia majora and minora become thickened, protruding and wrinkled. The clitoris enlarges and the urethral meatus becomes more prominent. During the first two years after the menarche, anovulatory cycles are common.

Pubertal growth precedes the final fusion of the growth cartilage. At the beginning of puberty, there is an acceleration in the growth rate. The peak growth in height, better known as the pubertal growth spurt, occurs during stage G3-G4 in males and G2-G3 in females. At the end of

puberty, gonadal steroids are responsible for the maturation and fusion of epiphyseal discs in both sexes, leading to the achievement of final height.

As previously mentioned, the time of onset of puberty can vary, and both precocious and delayed puberty are pathological conditions.

Precocious puberty (PP) is clinically defined by the appearance of the breast bud at less than eight years in girls, and by an early increase in testicular volume (cut-off 4 ml) before nine years in boys (Muir, 2006). PP is characterized by increasing growth rate, an early progression of secondary sexual characteristics and rapid bone maturation. There are two main forms:

Central Precocious Puberty (CPP) or Gonadotropin Dependent Precocious Puberty (GDPP). This involves early activation of the HPG axis that mimics a physiological pubertal progression. In males above all, it is associated with central nervous system (CNS) injuries such as tumours, and hence an MRI is indicated in all male subjects, while in females it is only indicated in cases of pubertal development occurring before six years of age, progressive PP, or neurologic symptoms such as headache, visual impairment or seizures (Carel & Léger, 2008);

Peripheral Precocious Puberty (PPP) or Gonadotropin Independent Precocious Puberty (GIPP). This is due to abnormally high production of sex steroids from gonads or adrenal glands (e.g. Leydig cell tumours, hCG-secreting tumours and ovarian tumours). It involves early development of secondary sexual characteristics and suppression of gonadotropin release by the pituitary gland.

Delayed Puberty (DP) is defined as the absence of breast development at 13 years old for females, and the absence of testicular volume increase over 4 mL at 14 years old for males (Palmert & Dunkel, 2012). The most common cause is a constitutional delay of growth and puberty (CDGP), which can be considered as a common normal variant of pubertal timing. This is more common in boys than in girls. The bone age (BA) is generally lower than the chronological age by 1 or 1.5 years, and height is lower than normal or within the lower limits of the range considered normal

for that age. The Growth rate is usually normal. CDGP has a strong genetic basis, as more than 75% of patients have a family history of delayed puberty (Wehkalampi et al., 2008). The remaining cases are considered idiopathic.

A major challenge is the differential diagnosis between CDGP and congenital hypogonadotropic hypogonadism (cHH). Recent works provide clinical and laboratory parameters that may be helpful for the differential diagnosis, starting with the possible presence of cryptorchidism, micropenis and several congenital anomalies typically associated with cHH, such as anosmia, renal agenesis, bimanual synkinesis, cleft lip and/or palate, congenital hearing impairment and optic nerve hypoplasia (Maione et al., 2018). As regards genetics, even though there seems to be an overlapping between the two conditions, as some genes seem to be shared (e.g., TAC3 and GNRHR), it has been recently demonstrated that the genetic patterns of patients with CDGP and control population are very similar (Young et al., 2019; Howard & Dunkel, 2019). The identification of new target genes will help in discriminating the two conditions with greater certainty.

The use of GnRH challenge to differentiate cHH from CDGP is highly debated. Broadly, CDPG produces a greater increase in gonadotropin levels, while the response is lower or absent in the case of cHH (Harrington & Palmert, 2012). Actually, the hypothalamus-pituitary reserve of subjects with cHH is extremely variable, and this often makes the GnRH test not conclusive in the differential diagnosis. Some studies evaluated the diagnostic power of both the GnRH test and the dosage of Inhibin B (Mosbah et al., 2020; Coutant et al., 2010). In particular, Mosbah et al. demonstrated that, in both cHH and CDGP patients, the increase in LH after GnRH challenge was very variable and it was correlated to testicular volume in subjects with cHH. Moreover, the authors showed that 47% of cHH subjects had a peak of LH comparable to CDGP patients, but no subjects with CDGP presented concentrations of LH below 4.0 IU/l, differently from 53% of patients with cHH. Finally, Coutant and co-authors studied the discriminating capability of the

basal Inhibin B blood level, and they concluded that in subjects with genital stage 1 (testis volume3 ml) there was a sensitivity and specificity of 100% for levels of Inhibin B equal to 35 pg/ml or less. On the contrary, these parameters lowered considerably when switching to genital stage 2 (testis volume 3-6 ml). Probably the combination of GnRH challenge, basal Inhibin B testing with clinical and genetic aspects can provide the best and most reliable results in discriminating between CDGP and cHH.

CONCLUSIONS

The role of numerous inhibitory and stimulatory neuromodulators in the onset of puberty is now well established. Most of them operate upstream of the so-called KNDy system, which definitively activates the GnRH pulse generator. The perfect balance between inhibitory and stimulating factors is crucial for the correct timing of puberty, with inhibitory factors predominating before puberty, and stimulating factors at its onset. The function of these refined neurophysiological mechanisms can be disrupted by many factors, especially EDs. It is also very important to diagnose early changes in the onset of puberty, whether precocious or delayed, because of the possible psychological and clinical short and long-term consequences.

CONFLICT OF INTEREST

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Table 1

Characteristics of mediators involved in the HPG axis function.

MEDIATOR	ENCODING GENE	RECEPTOR	KIND OF ACTION	ROLE ON HPG AXIS FUNCTION
Kisspeptin	KISS1, OMIM 603286	GPR54	Excitatory	GnRH secretagogue
NKB	TACR3, OMIM 162330	NK3R	Excitatory	GnRH secretagogue
Dyn	PDYN, OMIM 131340	KOR	Inhibitory	Inhibiton of kisspeptin secretion
Glutamate	----------	NMDA receptor	Excitatory	Promotion of GnRH pulsatility
GABA	----------	GABA A, B, C	Inhibitory	Inhibiton of GnRH pulsatility
Neuropeptide Y	NPY, OMIM 162640	Y1, Y2, Y4, Y6	Inhibitory	Regulation of GnRH suppression
RFRP-3	RFRP, OMIM 616984	NPFFR1	Inhibitory	GnRH neuronal inhibition
MKRN3	MKRN3, OMIM 603856	MKRN3 receptor	Inhibitory	Inhibiton of GnRH secretion
Neuroestradiol	----------	ESR1	Inhibitory	Negative inhibitory action; ESR1 deletion causes early kisspeptin and/or glutamate activation
Leptin	LEP, OMIM 614962	LEP-R	Permissive role	Leptin deficiency reduces KISS1 expression
Ghrelin	GHRL, 605353	GHS-R	Inhibitory	Inhibiton of GnRH and LH secretion
ACAP	ADCYAP1, OMIM 102980	ADCYAP1R1	Inhibitory or excitatory	stimulatory or inhibitory effect to gonadotropins' release

Abbreviations: GPR54, G-protein coupled receptor; GnRH, Gonadotropin-releasing hormone; NKB, Neurokinin B; TACR3, Tachykinin 3 gene; NK3R, Neurokinin 3 receptor; Dyn, Dynorphin; PDYN, Prodynorphin gene; KOR, κ-opioid receptor; NMDA, N-Methyl-d-aspartate; GABA, γ-aminobutyric acid; NPY, Neuropeptide Y gene; Y1, Y2, Y4, Y6, Neuropeptide Y receptor type 1, 2, 4, 6; RFRP-3, RFamide-related peptide 3; NPFFR1, Neuropeptide FF receptor 1; MKRN3, Makorin ring finger protein 3; ESR1, Estrogen receptor 1; LEP, Leptin gene; LEP-R, Leptin receptor; GHRL, ghrelin and obestatin prepropeptide; GHS-R, Growth hormone secretagogue receptor; ACAP, pituitary adenylate cyclase-activating polypeptide; ADCYAP1, Adenylate Cyclase Activating Polypeptide 1; ADCYAP1R1, adenylate cyclase activating polypeptide 1 receptor 1

Table 2

Main properties and hypothetical role on the HPG axis of the most common EDCs.

EDCs/GROUP	ABBR.	ORIGIN	OVERALL PATHOLOGICAL EFFECT	EFFECT ON HPG AXIS	REFERENCES
Bisphenol A/Bisphenols	BPA	Plastic bottles, epoxy resins, polycarbonate plastics and plastic toys	Estrogenic	The exposure of adult female mice to BPA disrupts the HPG axis, by enhancing AVPV-kisspeptin expression and release	Wang et al., 2014
Dichlorodiphenyl-Dichloroethylene/ Organochlorides	DDE	Pesticides (and contaminated fish, soil products and water)	Anti-androgenic, estrogenic	p,p'-DDE, a potent antiandrogen, increases secretion of GnRH and consequently of LH and testosterone	Martin et al., 2002
Per-and poly-fluoroalkyl substances/ Fluorosurfactant	PFAS	Commercial household products and electronics manufacturing. Contaminated fish, soil products and water	Anti-androgenic, anti-estrogenic	There are no studies available	----------
Phthalates/ Plasticizers	---------	Cosmetics and personal care products, medical devices, PVC products and toys	Anti-androgenic	The female rats exposure to high doses of DEHP results in a significant reduction of the Kiss1 mRNA expression in the anteroventral periventricular but not in the arcuate nucleus	Zhen et al., 2020
Polychlorinated Biphenyls/ Organochlorides	PCBs	Flame retardants, pesticides and contaminated fish, soil products and water	Variable (estrogenic, antiestrogenic or antiandrogenic)	Pre-natal exposure to PCDD/Fs (dioxins) and PCBs results in an alteration of the HPG axis, which occurs with a reduction of testosterone in females and estradiol in males	Cao et al., 2008