A wider perspective on puberty

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Abstract

The mechanisms that control the onset of puberty remain within the purview of the neurobiologists who first recognised the concept of removal of a restraint factor to permit reawakening of puberty. The discovery of a ligand-activated G protein receptor-signalling pathway upstream of the GnRH pulse generator adds further weight to the role of the hypothalamus in the central regulation of puberty. The physical pointers to puberty are the mainstay of assessing timing and tempo in a clinical setting but non-invasive and indirect methods of assessment only are appropriate for population studies in normal children. That puberty can be regarded as a sensor recognises the observation of secular changes in pubertal timing and perhaps qualitative aspects of tempo. The influences on the sensor include marked changes in nutrition and an environmental exposure to low-dose chemical mixtures interacting with a polygenic background.

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Keywords: GnRH; Pulse generator; Restraint; Tempo; Secular; Environment

1. Introduction

Puberty remains an enigma despite being three decades on from the first in the line of six major conferences devoted to deciphering the code, which triggers puberty in primates. Indeed, how neuroendocrine and genetic mechanisms control sexual maturation at puberty has been dubbed one of the great mysteries of human biology (Seminara et al., 2003). In a similar vein, it has been said that puberty results from the awakening of a complex neuroendocrine machinery in which the primary mechanism is still unclear (Parent et al., 2003). At least neurobiologists and biomedical scientists can take heart from not being alone when so tantalisingly close to understanding the origins of puberty, as it is not unlike how astrophysicists currently find themselves being able to measure discernible radiation so close to the ‘big bang’.

The practising clinician is interested in childhood growth translated into a pubertal growth spurt consequent upon factors that trigger the re-awakening process. Why should this occur on average at around 10–11 years of age in the human? Being incapable of understanding this fundamental process in normal biology makes it doubly difficult to understand why some children enter puberty for no apparent reason early (usually girls) and also for no apparent reason, some enter puberty late (usually boys). It is now necessary to take a wider perspective on puberty in recognition of results from epidemiological studies indicating that factors such as nutrition and the 80,000 or more chemicals in the environment to which humans are exposed may be playing a part in the apparent operational changes in the timing of puberty. Yet, genes remain important, accounting for about 75% of the variance in the timing of puberty.

This paper briefly reminds readers of the somatic signs of puberty which are key to assessing not only the individual who displays an aberrant timing but also in defining pubertal stages from large population studies. The word tempo is freely used by puberty researchers, primarily a musical term whose partners will be used in this paper to illustrate in a theatrical sense some of the variants of puberty encountered in clinical practice. This is placed in the context of some evidence to suggest an earlier onset of puberty in some parts of the world, perhaps reflecting environmental influences. Nevertheless, what has stood the test of time since the first Puberty conference in 1972 is the key role of a hypothalamic GnRH pulse generator mediating the biological switch for puberty. The molecular machinery governing this pulse generator now includes a G-protein coupled receptor and its ligand whose discovery and application in physiological and pathophysiological studies firmly cements the central role of GnRH in the control of puberty.

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2. **Pointers to puberty**

The landmark studies of Marshall and Tanner (1969) which classify puberty in five stages in girls and boys according to somatic changes in breast, pubic hair and genital development still remain nearly 50 years later the template upon which most anthropometric analyses of puberty are based. Fig. 1 illustrates a distillate of the observations which are most germane when considering the state of puberty both in an individual and in a population. Stage B2 is the start of puberty in girls and predates by stage B1 which denotes the infantile and early childhood phase of no breast development once the effect of transplacental passage of maternal estrogens has abated. A remarkably sensitive bioassay of estrogens was the demonstration of an increase in the number of stratified squamous epithelial cells on a vaginal smear which predates the onset of B2 (Marshall, 1975). This test clearly has no place nowadays in the clinical assessment of puberty. The equivalent to stage B2 in boys is an increase in testicular volume which is generally accepted to be a change from 3 to 4 ml as indicative of puberty starting (G2). While a useful and key sign used in clinical practice when assessing, for example, a boy with delayed puberty, it is impractical to use in population studies of puberty.

Fig. 1 refers to the phases between B2–B5 and G2–G5 as the tempo of puberty. A multitude of processes occur during this interval which typically lasts 3–4 years. Included is the pubertal growth spurt and in girls, menarche. These are pointers which are possible to record with reasonable accuracy in large population studies. There is no easily noted time equivalent to menarche in boys. Spermarche as defined by the first nocturnal emission is an impractical measure for epidemiological studies but the onset of spermaturia as measured in an early morning urine sample can be useful in studying changing patterns of male puberty (Schafer et al., 1990). Staging of pubic hair is also incorporated in to the Tanner classification and is generally a useful marker in both sexes. However, some growth of sexual hair on the pubes, scrotal, perineal or labial skin may develop long before signs of gonadotrophin-dependent puberty. This is the result of an increase in adrenal androgen production, a physiological event termed adrenarche for which the mechanism remains unclear (Havelock et al., 2004). Pubic hair alone should not be used as a marker of the onset of puberty.

3. **Timing and tempos of puberty**

Secular trends in puberty have long been recognised with improved nutrition and reduced childhood infection generally considered to be the explanation for a reduced age of menarche during the past couple of centuries. Now it has been proposed that the onset of puberty is occurring earlier than it did about 50 years ago if comparisons of puberty stages are made with the Tanner standards. Much of this evidence is based on a large cross-sectional study of 17,000 girls conducted in an office setting in the USA, the Pediatric Research in Office Settings (PROS) Study (Herman-Giddens et al., 2005). This and other relevant recent studies of puberty (Freedman et al., 2002; Chumlea et al., 2003; Demerath et al., 2004) highlight problems in the interpretation of epidemiological studies of puberty and are discussed in more detail in subsequent papers in this issue. Suffice it to say there may be a recent secular trend in the age of onset of breast development (B2) but the data for G2 are less reliable, the age of menarche has ‘stabilised’, there is still evidence of profound socioeconomic effects on menarchal age (mean ages of menarche 13.2 and 14.6 years in privileged versus underprivileged Black South Africans, respectively), menarche remains an earlier event in Blacks, there is a North-South gradient to menarche in Europe (in 1993, mean age in Finland 13.3 versus 12.3 years in Greece), and the events of puberty are generally associated with individual nutritional status as assessed by the BMI. A new phenomenon has emerged in recent years in the field of puberty epidemiology as illustrated by the observation of puberty occurring earlier in girls who have migrated from developing countries following adoption (Parent et al., 2003). This is clearly an environmental-based modulator of puberty timing and hence the thematic signature of puberty as a ‘sensor’ of gene: environment interactions being chosen for the 6th Symposium on Puberty.

Direct examination of children by health professionals to assess pubertal development is inappropriately invasive in population-based studies. Indirect methods have included verbal methods whereby individuals are asked has development started and to what degree (Petersen et al., 1988) or to make comparisons with their peer group (Kaiser and Gruzelier, 1999) and self-assessments of puberty stages based on pictorial representations (Duke et al., 1980; Neinstein, 1982; Hergenroeder et al., 1999). The correlation between ratings recorded by the individual and the study investigator was generally satisfactory for these studies. It is important to validate these various methods in large population studies if meaningful conclusions are to be reached about puberty now starting earlier. The increase in the prevalence of obesity has compounded the interpretation of B2 as a marker of puberty in girls, not only as assessed by visual inspection but even when direct palpation is the means of assessment. Serial measurements of growth allows peak height velocity to be calculated which, when used to define the age at which it occurs, is a reliable marker of pubertal maturation (Sandhu et al., 2006).

Another indirect measure of pubertal progression in boys is to determine the pitch or the speaking/singing fundamental frequency of the voice. Analysts in the musical world use a
Fig. 2. Changes in the male voice at puberty. (a) Correlation between Tanner (G) and Cooksey (C) staging of puberty. (b) Relation between stages of puberty as defined by Tanner and Cooksey stages and speaking fundamental frequency (A and B) and singing fundamental frequency (C and D) (Harries et al., 1997). Reproduced with permission.
six stage classification of pubertal voice development which is based on the singing range and tessitura, defined as the most comfortable modal singing voice range (Cooksey et al., 1984). The Tanner and Cooksey stages correlate well and in turn, with fundamental voice frequency (speaking and singing); see Fig. 2. The observations confirm that ‘voice breaking’ is a relatively late event in male puberty yet is often regarded by non-health professionals as the sign heralding the onset of puberty in boys. Applying an electrolaryngograph to the external larynx and asking the boy to ‘sing’ a musical scale or read a sentence provides a non-invasive indirect measure of male puberty which could be applied in population studies to assess whether secular changes have occurred. Since the word tempo is such an integral part of the musical and pubertal lexicons, perhaps a similar nomenclature could be applied to variations on the theme of puberty (Fig. 3). Thus the normal tempo of puberty may be in andante (moderate tempo) mode, whereas the boy commonly seen with constitutional delay in growth and development may be operating in lento (slow tempo) or even largo (very slow) fashion. Conversely, the musical term allegro con brio (quick tempo, with spirit, vigorously) conjures an apposite terminology to describe the child with precocious puberty of whatever cause, who is racing ahead in growth and skeletal development.

4. Genetics and the paradigm of puberty

The physiological paradigm of puberty in relation to gonadotrophin and sex steroid (testosterone) concentrations is depicted in Fig. 4. Central to this process is a period of ‘restraint’ during childhood, which, upon releasing via genetic and perhaps extrinsic mechanisms, allows puberty to be re-activated. The term re-activation implies evidence of a prior puberty which has a basis from the rise in gonadotrophin and concomitant steroid levels during fetal life. However, there is little direct evidence to indicate this is the result of an active GnRH pulse generator. In the male, the rise in gonadotrophins (initially placental hCG) and a subsequent marked increase in testosterone levels is crucial for differentiation of the internal and external genitalia and adequate descent of the testes to their scrotal position. Estrogens are not essential for differentiation of the female fetal genital tract so the concomitant increased gonadotrophin activity in the female fetus is devoid of any apparent consequence. Yet another surge in gonadotrophin and sex steroid secretion occurs in early infancy but what influence this phenomenon has, if any, on subsequent puberty, remains unclear. The ‘removal of restraint’ model of puberty is attested to by a rise in gonadotrophin secretion occurring, initially nocturnally, around the end of the first decade of life whether gonads are present or not. Thus, the rise in gonadotrophins as a result of activation of the GnRH pulse generator starts at the expected time of puberty in girls with Turner syndrome, boys with Klinefelter syndrome or with anorchia (Conte et al., 1975). These observations can be replicated in studies of agonadal monkeys which also demonstrate that the restraint on the GnRH pulse generator operating in prepuberty is more intense in the male (Plant and Barker-Gibb, 2004). It has been surmised that this sex dimorphism in pulsatile GnRH release is a legacy of the high levels of testosterone to which the male fetus is exposed. In turn, it may explain why delayed puberty (in lento or largo mode) is far more common in boys, whereas precocious puberty is more frequent in girls and generally idiopathic in nature.
A host of factors are involved in controlling the onset of puberty via the GnRH pulse generator restraint mechanism. These include GnRH, NPY, GABA, leptin, TGFα and their cognate receptors. It is always encouraging to see that progress has been made in the elucidation of puberty in the intervening years between Puberty Symposia. In this case, attention is focused on the discovery of the role of a G-protein coupled receptor and its ligand operating upstream of the GnRH pulse generator. GPR54 has been dubbed the gatekeeper to puberty (Fig. 5). This 398 amino acid receptor has 40% homology with galanin receptors, is expressed in the hypothalamus and pituitary but also in the placenta and pancreas where expression is highest (Colledge, 2004). The natural ligand for GPR54 is kiss-54 peptide, a derivative of a 145 amino acid peptide. The peptide is also called metastatin in view of its properties of inhibiting metastatic growth, first identified in a melanoma cell line. Injection of kiss-54 directly stimulates LH and FSH release via GPR54 activation of GnRH which can be abolished by prior treatment with a GnRH antagonist. Like its cognate receptor, kiss-54 is expressed in the placenta and serum levels are markedly increased from the eighth week of pregnancy onwards. What role this serves during pregnancy has not been established.

The quaint background to the derivation of kiss terminology is not entirely disconnected from discussion of the subject of puberty, a process so fundamental to fostering relationships and propagation of the human species (Fig. 6). That metastatin has suppressor functions is denoted by SS (suppressor sequence) in Kiss. Since work on this peptide was undertaken at the Hershey Medical Center in Pennsylvania, the researchers turned to their local chocolate industry for inspiration by referring to the word KISS coined by Hershey in 1907 whose spoken sound recapitulates the sound of chocolates being deposited during the manufacturing process. The illustrations of the Rodin sculpture and Warhol painting completes the pictorial use of poetic license (Fig. 7).

It is often the case that studying the consequences of natural mutations in key genes involved in growth and development provides valuable information about physiological mechanisms. Witness the profound impact studying mutations of the aromatase and estrogen receptor genes has had on understanding the maturation of the growth plate and completion of linear growth (Grumbach, 2004). In a similar vein, mutations in the GPR54 gene result in hypogonadotropic hypogonadism in humans (Seminara et al., 2003; Semple et al., 2005). Targeted disruption of Gpr54 in mice is associated with normal sex differentiation at birth but delayed sexual maturation thereafter. Genetic causes of hypogonadotropic hypogonadism now include not only mutations in GPR54 but also the KAL-1, FGFR1 and GnRHR genes. It is clear that the genetic control of puberty is polygenic in nature and analysis of candidate genes is certainly of considerable value in the study of pathological states such as hypogonadotropic hypogonadism. However, mutations in GPR54, for example, have not been found in boys with large related pubertal delay nor have associated polymorphic sequence variants in the gene been found (Lanfranco et al., 2005). The tempo aspect of puberty remains a tantalising biological phenomenon to address and...
requires an investigative strategy which extends beyond neurobiology at its core. It is in such a context that the topic briefs in this 6th Symposium are wide-ranging and have set out to demonstrate that puberty may indeed be a sensitive biomarker of intrinsic:extrinsic interactions operating within a changing environment.

5. Questions to ponder

The physical signs that herald the onset of puberty are familiar and are well documented for the individual. The timing and tempo of pubertal events are in a state of flux and only epidemiological studies of large populations that are ethnically and socially diverse can provide information about any secular trends. Direct examination, appropriate for an individual in a clinical setting, is not acceptable for studies of normal children. Validation of self-assessment methods of pubertal staging coupled with documentation of biological parameters allied to puberty (growth spurt, voice breaking) needs to be undertaken to improve the reliability of results from epidemiological studies. The widespread increase in childhood obesity and the inextricable link between nutrition and puberty means that any secular change in puberty manifestations may occur over a shortened time span.

Neurobiology remains a central plank of puberty research to which has been added knowledge of a cell signalling mechanism that re-affirms the key role of the GnRH pulse generator. Further studies are needed to elucidate whether variants in factors that control the puberty ‘re-awakening’ process may explain the quite profound alterations in timing and tempo of puberty observed in man. It is in this context that environmental factors such as chemicals with endocrine modulating effects may be relevant. The abundance of evidence from animal studies that such chemicals can be modulatory has yet to be even part replicated in humans. Continued endocrine, genetic and imaging studies in the child with parameters of puberty outwith the accepted age range will ensure further progress in delineating the mechanisms of pubertal pathophysiology and thereby, the introduction of novel modes of treatment.

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