The neuroendocrine timing of puberty

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Abstract

Puberty is the attainment of fertility, a process encompassing morphological, physiological and behavioural development. The increased hypothalamic secretion of the gonadotrophin-releasing hormone decapeptide (GnRH) is essential for the activation of the pituitary–gonadal axis at puberty. The GnRH secretory network initially develops and is temporarily active during species-specific periods of fetal/neonatal development, so puberty is the secondary reactivation of an existing system. From a neurobiological perspective, the timing of puberty is therefore a function of changes in the neural systems controlling GnRH release. The large variability between individuals in the onset and progression of puberty indicates that the timing of puberty is not simply a function of chronological age. Rather, the neurotransmitter and neuromodulatory systems that impact upon the GnRH secretory network convey information about metabolic fuels, energy stores and somatic development and, for many species, information about season and social environment. The clear links demonstrated between metabolic fuel availability and reproductive function in many animal models provides evidence that the earlier onset of pubertal development observed in girls in certain US study populations is likely to relate to the increasing prevalence of overweight and obesity in adolescents.

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Gonadotrophin-releasing hormone (GnRH-I) secretion is essential for puberty

In neuroendocrine terms, puberty is the reactivation of gonadotrophin-releasing hormone (GnRH) secretion (Fig. 1). Gene duplication has led to multiple forms of this hormone being produced in vertebrates (Fernald & White 1999), but the profound infertility observed in the *hypogonadal* (*hpg*) mouse in which the GnRH-I gene is mutated such that it is not translationally competent, elegantly demonstrates the critical importance of this particular gene product in mammals (Cattanach *et al.* 1977, Charlton 2004). It was first demonstrated over two decades ago that treatment of prepubertal primates with appropriate pulsatile patterns of GnRH alone induces puberty (Wildt *et al.* 1980). The necessary and sufficient role of GnRH-I (henceforth referred to as GnRH) in causing puberty in mammals has been further demonstrated by the lack of gonadotrophin synthesis and secretion, and the retardation of reproductive development or the loss of reproductive function in adulthood when the action of GnRH has been blocked by immunoneutralisation, treatment with GnRH antagonists, or by treatment with agonists which paradoxically down-regulate the secretory activity of gonadotrophs in the pituitary gland. Conversely, treatment of infertile *hpg* mice with synthetic GnRH or with grafts of wild-type fetal

hypothalamic tissue containing normal GnRH neurons, or by gene therapy induces spermatogenesis in males (Mason *et al.* 1986). Such grafts also induce ovarian maturation in females such that ovulation can be induced by exposure to sexually active males and pregnancy established (Gibson *et al.* 1984), confirming the critical role of GnRH in reproductive maturation. Thus, the ultimate attainment of fertility, reflecting morphological, physiological and behavioural development, is a consequence of the increased synthesis and release of this decapeptide with unusual terminal modifications. It is perhaps surprising, given the absolute requirement for increased GnRH secretion to cause reproductive maturation, that this remarkable neuroendocrine output is produced by less than 2000 neurons in the mammalian brain (Ebling & Cronin 2000).

Development of the GnRH secretory system

Another remarkable feature of this GnRH secretory system is that the key population of GnRH neurons originates in the embryonic period outside of the central nervous system in the medial olfactory placode (Tobet *et al.* 2001). These cells subsequently migrate from the nasal region into the brain, such that the neuronal cell soma which

Figure 1 The timing of puberty is a function of the central control of GnRH secretion, reflecting the integration of multiple internal and external cues acting upon a genetically determined process. Some major disorders of the timing of puberty are indicated that reflect dysfunction at different levels of the hypothalamo–pituitary–gonadal axis. E₂, oestradiol; FSH, follicle-stimulating hormone; GnRH-R, gonadotrophin-releasing hormone receptor.

constitute the GnRH secretory system in the adult mammal are dispersed in a rostro-caudal continuum through the basal forebrain from the medial septum to the medio-basal hypothalamus. The majority of cell bodies are found in rostral areas, particularly the medial septum, diagonal band of Broca, and in the preoptic area (POA) at the level of the organum vasculosum of the lamina terminalis (OVLT). In primates and man, a proportion of the GnRH-immunoreactive perikarya are also located more caudally in the retrochiasmatic area, and in the mediobasal hypothalamus and arcuate nucleus (Song *et al.* 1987, Dudas & Merchenthaler 2003). Retrograde tracing studies reveal that the great majority of GnRH neurons extend axonal projections to the capillary beds of the OVLT and median eminence (Witkin 1990), although extensive branching also results in innervation of midbrain sites where GnRH has been hypothesised to facilitate certain reproductive behaviours. Such observations lead to the view that the major role of GnRH, at least in the postnatal period, is via secretion into the pituitary portal circulation ultimately to regulate pituitary gonadotroph function.

This recent understanding of the development of the GnRH secretory system provides an explanation for Kallmann's syndrome (Fig. 1), where the timing of puberty may be grossly delayed or absent resulting in complete infertility (MacColl *et al.* 2002). In the great majority of cases the hypogonadotropic hypogonadism is linked with anosmia, the complete lack of a sense of smell, or hyposmia. This association between olfactory deficits and reproductive maturation was first described by Maestre de San Juan in 1856, who reported an autopsy of a 40-year-old man with a micropenis, very undersized cryptorchid testicles, and absent olfactory bulbs (Munoz & Dieguez 1997). Kallmann (1944, cited in Quinton *et al.* 1996) later reported that this association occurred within families, establishing its genetic basis, although it can also occur as a sporadic disorder. The lack of GnRH secretion underlying the hypogonadotropism is a consequence of incomplete migration of GnRH neurons from the olfactory placode to the brain, which, in turn, is linked to dysgenesis of the olfactory bulbs. The syndrome is far more prevalent in males than females, by a factor of 5:1. This

results from a common form in which there is X-linked inheritance, and the underlying mutation is in the KAL-1 gene which encodes a protein with homology to the cell surface adhesion molecule N-CAM (MacColl *et al.* 2002). Presumably the failure of GnRH neurons to migrate appropriately in such individuals reflects the non-permissive nature of the neural substrate over which migration along the olfactory nerve takes place.

Developmental changes in GnRH gene expression

The observation that the neural substrate for puberty develops so far in advance of the onset of puberty raises the question of why the GnRH secretory system does not become active and drive puberty as soon as it has developed. The idea that GnRH gene expression is arrested, and thus the synthetic capacity of the GnRH secretory network is limiting, has been addressed using a variety of experimental approaches in several different species. However, there is little evidence that changes at the level of GnRH gene expression are limiting the timing of puberty. For example, in the male mouse, GnRH mRNA abundance as assessed by *in situ* hybridisation increases significantly between the day of birth and five days of age, but then remains high through to adulthood (Ford & Ebling 2000), that is, there are no further increases at times when increases in gonadotrophin secretion and testosterone concentrations occur. Likewise, in a study of C57Bl/6 mice in which cytoplasmic GnRH mRNA and nuclear primary transcript levels were separately assessed by RNase protection assays, a gradual developmental trend in cytoplasmic GnRH mRNA was reported, but most of the increase occurred by postnatal day 20 in females, and before postnatal day 28 in males (Gore *et al.* 1999). Moreover, the major increase in levels of nuclear GnRH transcripts as determined by RNase protection assay occurred between postnatal day 0 and postnatal day 7 in both sexes (Gore *et al.* 1999). These findings in the mouse are complemented by many studies in the rat. In an *in situ* hybridisation study in the male rat, no increases in GnRH mRNA levels were detected at the time of the pubertal increase in plasma testosterone, that is, after 25 days of age (Wiemann *et al.* 1989). However, significant increases were observed at earlier ages, as determined by solution hybridisation RNase protection assays (Gore *et al.* 1996) or by PCR-based assays of hypothalamic extracts (Dutlow *et al.* 1992). Differences in the choice of time points studied make it difficult to compare directly the various studies in rats and mice, but in general the major developmental increase in GnRH gene expression in the mouse occurs at a younger age than in the rat, consistent with the earlier increases in gonadotrophin secretion in mice.

In a more limited number of studies in primates comparing GnRH mRNA abundance or hypothalamic GnRH content between prepubertal and postpubertal stages, there is little evidence for changes in the capacity to synthesise or

store GnRH (Fraser *et al.* 1989, Vician *et al.* 1991). Thus, the common finding across many mammalian studies is that increases in GnRH gene expression and translation precede the increases in gonadotrophin secretion which drive gonadal maturation (reviewed in Plant 2001). It seems highly unlikely that insufficient expression of the GnRH gene is limiting the timing of pubertal development. Moreover, any changes in mRNA abundance occurring at the time of puberty are more likely to reflect post-translational mechanisms, for example increased stabilisation of the mRNA, rather than changes in the primary transcription rate (Gore & Roberts 1997).

Puberty as a reactivation of GnRH secretion

The studies briefly summarised above demonstrate that the anatomical development of the GnRH secretory system occurs relatively early in life, and that the synthetic capacity is present well before puberty in that GnRH mRNA expression reaches adult levels. In some species the GnRH secretory system is undoubtedly active in the mid-late fetal period or neonatal period. Studies in the fetal sheep reveal that luteinising hormone (LH) is released in a pulsatile manner during mid-late pregnancy, and the frequency of pulses increases if the fetal testis is removed (Mesiano *et al.* 1991). It is highly likely that the episodic release of LH reflects pulsatile release of GnRH from the fetal hypothalamus; indeed pharmacological blockade of GnRH action in fetal lambs results in a substantial reduction in testicular volume in later life (Brooks *et al.* 1995), evidence that activation of GnRH secretion early in life is important in testicular development. In male primates including humans there are periods of activation both in the fetal period and in the very early neonatal period (Plant 1985, Mann & Fraser 1996). These early periods of activation of the GnRH secretory system may be important for masculinisation of the brain, development of subsequent sexual behaviour, and control of Sertoli cell proliferation in the testis. In female primates, it seems that a low level of activity of the GnRH secretory system is present throughout the juvenile period (Pohl *et al.* 1995). Birth is an arbitrary event in the development of an organism, so the periods of activation of GnRH secretion occurring prenatally in precocious species such as the sheep may be equivalent to the postnatal periods of activation in altricial species such as the mouse. In general, the rapidity of growth and development in mammals with a short life span, for example laboratory strains of rats and mice, is such that the initial fetal/neonatal period of activation is continuous with and indistinguishable from the subsequent pubertal activation of the hypothalamo–pituitary–gonadal axis, so there is no juvenile hiatus as in larger mammals with longer life spans. However, some small mammals can display periods of juvenile reproductive quiescence, particularly when the reactivation of GnRH secretion is timed by

environmental cues such as changing photoperiod. For example, Siberian hamsters will rapidly grow and initiate puberty when raised in long photoperiods, but maintain a juvenile hiatus in development when exposed to short photoperiods signalling the onset of winter (Ebling 1994, Ebling & Cronin 1998).

Since the synthetic capacity of GnRH neurons is not limiting for puberty to proceed, and a period of endogenous GnRH secretion has already occurred earlier in development, puberty can be viewed as a *reactivation* of GnRH secretion. A great deal of attention has been paid to the issue of whether the GnRH secretory system is actively inhibited during the prepubertal period or is simply dormant, waiting to acquire stimulatory inputs. The concept of the 'gonadostat' has arisen; this suggests that the low levels of androgen/oestrogen released by the prepubertal testes and ovaries exert negative feedback effects that inhibit GnRH release prior to puberty. Whilst there is no doubt that such homeostatic feedback mechanisms play an essential role in the regulation of the reproductive axis, it is clear that the decrease in GnRH secretion in the neonatal primate, and the primary activation of GnRH secretion in the juvenile period are processes that are independent of gonadal steroid feedback. This is elegantly demonstrated by the decrease in gonadotrophin secretion after the neonatal period and the subsequent increase in the juvenile period in rhesus monkeys that have been castrated at birth to remove any confounding influence of gonadal steroid feedback (Plant 1985). However, the juvenile increase in gonadotrophin secretion in such agonal animals occurs earlier than in gonadally intact monkeys, which demonstrates that steroid feedback mechanisms also play a role in the later stages of pubertal maturation (Rapisarda *et al.* 1983). In the juvenile period there is a progressive decrease in sensitivity to the feedback effects of androgens and oestrogens. The high sensitivity at the start of the pubertal process explains why the initial menstrual/oestrous cycles are often anovulatory, or consist of short luteal phases or inadequate luteal function (Rapisarda *et al.* 1983, Foster 1994). Perhaps rather than considering the 'gonadostat' as a causal mechanism for puberty, it should be viewed as the necessary readjustment of gonadal steroid feedback loops which allows full ovarian and testicular activity to develop.

The capacity for this reactivation exists throughout the quiescent prepubertal period, as electrical and neurochemical stimulation of the hypothalamus have both been shown to release LH, and by inference GnRH. For example, treatment with the glutamate agonist N-methyl-D-aspartate (NMDA) rapidly induces LH secretion in a wide range of prepubertal species including primates, sheep and rodents (Plant *et al.* 1989, Ebling & Cronin 1998). Concurrent treatment with GnRH receptor antagonists blocks the effect of NMDA indicating a likely action on GnRH release rather than an action directly upon the pituitary gland (Lincoln & Wu 1991). Moreover, in adult sheep, NMDA has been shown to increase

GnRH concentrations in the portal capillaries draining the median eminence (Viguie *et al.* 1995). Such evidence that the GnRH secretory system can be driven by exogenous activation supports the view that the endogenous stimulation of GnRH release is the rate-limiting step for puberty. The GnRH neuron may therefore be viewed as a very passive element in the pubertal process in that its secretion is entirely dependent upon inputs from higher centres.

Neural inputs to GnRH neurons

If from a neurobiological perspective the timing of puberty is a function of the activation of the GnRH secretory system by higher order mechanisms, then the precise identification of neural inputs to this system is paramount. Neuroendocrine studies in adult mammals employing pharmacological activation or blockade, in combination with neuroanatomical and electrophysiological techniques, have provided evidence that all the major monoamine and amino acid neurotransmitters can ultimately influence GnRH secretion (see Brann & Mahesh 1997, Terasawa & Fernandez 2001 for recent reviews). Likewise, a multitude of neuropeptides and systemically produced steroidal and peptide hormones can also ultimately modulate GnRH release. Such studies have not unequivocally demonstrated which pathways act *directly* upon GnRH neurons, or which inputs change during pubertal development, nor the relative importance of the plethora of different pathways. One problem in identifying inputs to GnRH neurons reflects the unusual development and organisation of the GnRH secretory system. The widespread and scattered distribution of individual cells has not been amenable to traditional electrophysiological techniques, but the creation of transgenic mice in which green fluorescent protein (GFP) is driven by a murine GnRH promoter has allowed recordings from identified GnRH neurons in hypothalamic slices (Spergel *et al.* 1999, Suter *et al.* 2000). Whole-cell current clamp recordings reveal that GFP-tagged GnRH neurons fire spontaneous action potentials, although it does not appear that the firing patterns resemble the circoral frequency of pulsatile GnRH release *in vivo*. Patch clamp studies reveal that all GFP-GnRH neurons are highly responsive to γ -aminobutyric acid (GABA), and all show much lower amplitude responses to L-glutamate (Spergel *et al.* 1999). Consistent with these findings, single cell RT-PCR of forebrain cells identified as GnRH neurons on the basis of their morphology and subsequent amplification of GnRH mRNA has revealed the presence of a variety of GABA and glutamate receptor subunits within putative GnRH neurons (Herbison *et al.* 2001). This evidence for direct responses to GABA is consistent with studies indicating a role for GABA in both the initial development of the GnRH secretory system and in the differential regulation of GnRH secretion across puberty (Terasawa & Fernandez 2001). Likewise, the evidence for

direct glutamate responses via both AMPA and NMDA receptor complexes is consistent with the view that glutamate plays an important role in secretion of GnRH and in stabilisation of GnRH mRNA (Brann & Mahesh, 1997, Hrabovszky *et al.* 2004). Spergel and colleagues (1999) recorded from GFP-GnRH neurons in tissue slices from mice ranging in age from 1 week to 6 months, but reported no differences in GABAergic or glutamatergic responses at different stages of reproductive development. This finding is consistent with the view that GnRH neurons are mature from very early in development and that increased secretion reflects changing signals to the GnRH secretory system rather than maturational changes within GnRH neurons themselves, for example changes in the expression of receptor subunits.

Immunoelectronmicroscopy studies suggest that the density of synaptic appositions on GnRH-immunoreactivity (GnRH-ir) neurons is very much lower than that on surrounding neurons of unidentified phenotype (Lehman *et al.* 1988). A study comparing the density of synaptic inputs to GnRH neurons in seasonally anoestrus and ovulating sheep detected a twofold increase in synaptic appositions onto the cell soma and dendrites during the breeding season (Xiong *et al.* 1997). No such changes were detected in non-GnRH neurons in the surrounding preoptic area and the changes persisted in steroid-clamped ovariectomised ewes, indicating that the synaptic changes were not secondary to changes in circulating sex steroid concentrations (Xiong *et al.* 1997). It is clear that the annual transitions in fertility in seasonally breeding mammals (recurrent annual puberty) share many characteristics with the pubertal process in non-seasonal mammals (Ebling & Foster 1990), so a fascinating interpretation of the seasonal study is that puberty reflects the acquisition of stimulatory synaptic inputs to the GnRH secretory system. Such a 'structural plasticity' hypothesis is certainly consistent with the evidence presented above that GnRH neurons are passive elements in the pubertal process, and that puberty results from changes at a higher level. Rather few ultrastructural studies have been carried out to compare synaptic innervation of GnRH-immunoreactive neurons in a developmental context, perhaps reflecting the inherent difficulty of the necessary techniques. In support of the 'structural plasticity' hypothesis, a study comparing gonadally intact prepubertal (29-day-old) and adult (3-month-old) male rats reported an increase in synaptic density on GnRH cell soma (Witkin & Romero 1995), but studies in primates have been equivocal. One study in castrated juvenile and peripubertal male rhesus monkeys did not detect any developmental difference in the density of synapses onto GnRH dendrites (Perera & Plant 1997), and reported a small decrease in synaptic density on GnRH perikarya in the mediobasal hypothalamus (Perera & Plant 1997). A study in gonad-intact female rhesus monkeys did not detect any differences in synaptic densities between pubertal and adult monkeys (Witkin *et al.* 1991).

A fair comparison of these ultrastructural studies across species is not possible because of a myriad of differences relating to age, developmental status, sex, steroidal milieu, particular localisation of GnRH perikarya, and methods of analysis, but it would be premature to reject the 'structural plasticity' hypothesis on the basis of very limited studies in primates. Moreover, the focus of the above studies was on axosomatic and axodendritic synapses. Plasticity in glial ensheathment of GnRH terminals in the median eminence (Witkin & Romero 1995), or even of synaptic input at this level (Durrant & Plant 1999), might also alter GnRH release at puberty.

Immunocytochemical colocalisation of low amounts of receptor proteins within GnRH neurons has provided further evidence of neuropeptide pathways and hormonal signals that might modulate GnRH release. GnRH neurons in adult mammals express receptors for a number of peptides involved in hypothalamic regulation of energy balance, for example neuropeptide Y (NPY) (Li *et al.* 1999), and vasoactive intestinal peptide (VIP) (Smith *et al.* 2000), and receive appositions from metabolic peptidergic systems including CART (Cocaine and amphetamine-regulated transcript; Leslie *et al.* 2001) and orexin (Iqbal *et al.* 2001a). Fetal GnRH neurons have been shown to express growth factor receptors (e.g. brain-derived neurotrophic factor; BDNF) (Cronin *et al.* 2004), and recently expression of the GPR54 kisspeptin-receptor has been localised in GnRH neurons in the mouse (Messenger *et al.* 2005). The GPR54 receptor is of particular interest since dysfunctional mutations of this receptor result in blocked pubertal maturation in both mouse and man (Seminara *et al.* 2003).

The timing of puberty

Perhaps the main difficulty in identifying specific inputs to the GnRH secretory system relates to its highly integrative function. The timing of puberty is clearly not a simple function of chronological age. At the individual level, there is a substantial heritability of the timing of puberty (Palmert & Hirschhorn 2003 for review), but many internal and external factors impinge upon the genetic blueprint (Fig. 1). Both the onset of puberty and subsequent post-pubertal reproductive function are affected by a huge number of sensory inputs relating to both the internal and external environment. Information about growth, body fat/composition, diet, stress, annual change in photoperiod, circadian time, gonadal steroids, energy expenditure in locomotor activity and olfactory cues all must ultimately reach the mammalian GnRH secretory system (Fig. 1), so it is no surprise that a large number of neurotransmitters and neuropeptides have been implicated in signalling to GnRH neurons. Moreover, although much of the current interest in environmental pollutants and endocrine disrupting chemicals (EDCs) has focused on direct actions on testicular development and function (Fisher 2004), one cannot rule out the possibility that they may

also have actions within the brain upon GnRH synthesis and secretion (Fig. 1).

Many of the internal and external factors depicted in Fig. 1 have an effect on the timing of human puberty, so understanding how such information is transduced by the brain is currently of particular importance given certain lines of evidence in girls that the age of menarche is becoming earlier. One study in the USA (Herman-Giddens *et al.* 1997) collected data from over 17 000 adolescent girls who attended family health practitioners for reasons other than those related to growth or reproductive development. This study revealed earlier onsets of breast development, pubic hair growth and menarche relative to norms defined two decades earlier. This advance in the age of attainment of pubertal markers correlates with an increasing prevalence of overweight body condition in adolescents; for example almost 25% of adolescents in the USA were overweight in 1994 as compared with 15% in 1980 (Troiano & Flegal 1998). Many studies have used cross-sectional data to substantiate the association between obesity and early sexual maturation in girls (Adair & Gordon-Larsen 2001, Kaplowitz *et al.* 2001, Wang 2002). A recent longitudinal study provides further evidence for a link between body weight and onset of puberty, as those girls that had a higher body weight or body mass index earlier in childhood were more likely to initiate reproductive development at nine years of age than their peers (Davison *et al.* 2000). An outstanding review by Parent *et al.* (2003) provides a full analysis of the geographical prevalence of declining age of puberty, of the racial and environmental factors which might influence this, and of the question as to whether similar trends might exist in male populations, as some studies suggest a more complex relationship between early onset of fat deposition and pubertal maturation in boys (Wang 2002).

The correlation between body weight and onset of puberty has long been regarded as a causal relationship, encapsulated in the 'critical body weight' hypothesis (Frisch & McArthur 1974). The causality has been established in many animal models. Across a wide range of species, restricting body weight gain by means of limited nutrition delays the timing of puberty (l'Anson *et al.* 1990 for review). The body weight hypothesis has been refined as it became clear that body composition and, more specifically, body fat were better predictors of puberty than body weight *per se*. Identifying a correlation between the earlier onset of weight gain/body fat, and the earlier onset of puberty raises the key question of the mechanistic link between energy metabolism and the GnRH secretory system. The most contemporary view is that it is metabolic fuel (caloric) availability that is the key element determining reproductive function (Schneider *et al.* 2000); thus fat deposition is a correlate of the positive energy balance rather than the causal factor. This view stems from the rapidity with which increased energetic challenges (starvation, enforced activity) can suppress ovulatory cycles and reproductive behaviour in female mammals if

insufficient fat reserves are available to meet these demands (Bronson 2000, Schneider & Wade 2000).

The discovery of leptin in 1994 (Zhang *et al.* 1994) further complicates the metabolic fuels versus body fat debate. This adipocyte-derived cytokine is clearly an important element in energy homeostasis in that it negatively feeds back via receptors in the hypothalamus to reduce caloric intake and to increase caloric expenditure (Friedman & Halaas 1998). A role for leptin in the timing of puberty was hinted at by the observation that genetically mutant mice lacking leptin production (*ob/ob*) or leptin receptors (*db/db*) did not undergo normal puberty despite being grossly obese (Batt *et al.* 1982). Replacement administration of leptin to *ob/ob* mice induces reproductive development (Foster & Nagatani 1999 for review), and likewise leptin therapy has been shown to increase gonadotrophin secretion in an obese child congenitally lacking leptin (Farooqi *et al.* 1999). The observations in *ob/ob* mice, coupled with studies in which leptin treatment of prepubertal mice slightly advance the age of vaginal opening (Chehab *et al.* 1997), led to the concept that leptin was a trigger for puberty, signalling the presence of sufficient fat reserves to begin the energetically costly process of reproduction. However, subsequent studies in rats have failed to repeat the observation that leptin treatment can advance puberty in wild-type individuals, although it can overcome the delay in puberty induced by food restriction (Cheung *et al.* 1997). Measurements of circulating leptin in rodents and primates have failed to identify a specific rise at the time when increased gonadotrophin secretion is occurring (Ahima *et al.* 1998, Urbanski & Pau 1998, Plant & Barker-Gibb 2004). The situation in children is more complex. In girls there are gradual age- and body weight-related increases in circulating leptin concentrations (Clayton *et al.* 1997). In boys, leptin levels are considerably lower than in girls, but also rise until the early stages of puberty after which they decline (Demerath *et al.* 1999). This sex difference may represent stimulatory effects of oestrogens on leptin production and inhibitory actions of testosterone (Demerath *et al.* 1999). Collectively, these observations favour the view that leptin provides a permissive 'gate' for puberty rather than a causal 'trigger'. In human development, we must look for factors in addition to leptin to explain the timing of puberty, although in wild mammals experiencing highly variable food supply, sub-threshold circulating leptin concentrations might be an important signal to delay puberty in favour of processes necessary for immediate survival including increased appetite, reduced metabolic rate and thermogenesis.

It seems unlikely that leptin acts directly upon GnRH neurons since GnRH perikarya are mainly located rostral to the hypothalamic areas expressing leptin receptors (Mercer *et al.* 1996). However, a number of peptidergic cell phenotypes in more caudal areas of the hypothalamus do colocalise leptin receptors, for example NPY/agouti-related peptide (AgRP) and pro-opiomelanocortin

(POMC)/CART neurons in the arcuate nucleus, and melanin concentrating hormone (MCH) and orexin neurons in the lateral hypothalamus (Hakansson *et al.* 1988, Iqbal *et al.* 2001b). Moreover, leptin clearly regulates the activity of multiple hypothalamic peptidergic systems implicated in the control of energy metabolism (for example MCH, NPY, CART, orexin), many of which have been shown to extend rostral projections to areas implicated in the control of GnRH secretion (Parent *et al.* 2000). Given the profound hypogonadotrophism seen when GPR54 receptor signalling is dysfunctional (Seminara *et al.* 2003), an intriguing possibility is that direct metabolic regulation of the GnRH secretory system is via GPR54 receptors known to be expressed in GnRH neurons (Messenger *et al.* 2005). The finding that hypothalamic KiSS-1 mRNA abundance increases at the time of increased pubertal GnRH secretion in agonadal male and intact female rhesus monkeys is consistent with the hypothesis that the kisspeptin-GPR54 system contributes to the timing of puberty (Shahab *et al.* 2005). A demonstration that local production and release of ligands (kisspeptins) in the vicinity of GnRH neurons changes through pubertal development or in relation to energy balance is eagerly awaited.

Summary and future directions

Major advances have occurred in the last decade or so in understanding the timing of puberty. These include the discovery that GnRH-I neurons develop in the olfactory placode and migrate into the forebrain, the recognition that puberty is a reactivation of GnRH secretion, the identification of peripheral metabolic signals which may permit puberty to occur, and the identification of GPR54 as a key signalling pathway to GnRH neurons. Progress has also been made in identifying the neural mechanisms by which external environmental cues time puberty. These include the unravelling of the molecular basis of the circadian clock that underlies photoperiodic control of puberty in seasonal species (Lincoln *et al.* 2003 for review) and the identification of the structure of a class of pheromones in urine which regulate puberty via effects on vomeronasal neurons (Leinders-Zufall *et al.* 2000). The discovery of other populations of GnRH neurons in mammals expressing the GnRH-II gene (Latimer *et al.* 2000) is intriguing, but the functional significance, if any, is currently unclear. These advances generate further questions: what regulates the migratory pathways of GnRH neurons, and what controls axon outgrowth and targeting to the median eminence? What is the mechanism that causes GnRH secretion to decline between the neonatal and prepubertal phase of development in mammals with relatively long life spans? How do metabolic fuels and metabolic hormones such as leptin and other sensory inputs finally communicate to the GnRH neuron? How do GnRH neurons communicate with each other such that co-ordinated pulsatile release of GnRH

occurs? What is the function of GnRH-II? Some of these issues may be better addressed using the transgenic technologies which allow the identification and thus the recording, sampling and observation of GnRH neurons in living tissue, but in order to understand how internal and external cues time puberty it will also be important to study a variety of other mammalian models in which the relative importance of such inputs differs.

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