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The role and potential sites of action of thyroid hormone in timing the onset of puberty in male primates

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Abstract

Puberty in primates is first delayed by a neurobiological switch that arrests pulsatile GnRH release during infancy and then triggered, after a protracted period of juvenile development, by resurgence in intermittent release of this hypothalamic peptide. The purpose of this chapter is to review recent studies conducted in our laboratories to begin to examine the role of thyroid hormone (TH) in governing this postnatal development of pulsatile GnRH release in primates and therefore the timing of puberty in these species. The male rhesus monkey was used as the experimental model and TH activity was manipulated by surgical and chemical thyroidectomy on the one hand, and by thyroxine (T₄) and triiodothyronine (T₃) replacement on the other. Our results indicate that the resurgence in pulsatile GnRH release at the termination of the juvenile phase of development is dependent on a permissive action of TH. Whether this action of TH is mediated directly on hypothalamic centers regulating the pulsatile release of GnRH, or indirectly by circulating signals reflecting TH action on somatic development remains to be determined.

Keywords

thyroid hormone; somatic development; puberty; GnRH pulse generator; primates

Introduction

Puberty in primates is initiated by a resurgence of robust pulsatile GnRH release after a prolonged phase of juvenile (and childhood in man) development when the hypothalamic network responsible for the release of this hypophysiotropic hormone (the GnRH pulse generator, Plant, 1986) is held in check¹ by mechanisms that are poorly understood (Plant and Witchel, 2006). During early infancy, however, before the check on GnRH release is applied, circulating gonadotropin levels are elevated and, in infantile male primates, associated with blood testosterone levels in the adult range (Mann et al., 1984; Plant and

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¹The check or brake on pulsatile GnRH release during juvenile development is a conceptual check or brake. It may be occasioned by the loss of a stimulatory input or the addition of an inhibitory input or a combination of the two.

Witchel, 2006). Thus, the timing of puberty in primates may be viewed to be governed by two major postnatal switches; the first is activated during infancy and suppresses pulsatile GnRH release that results in a hypogonadotropic state that in turn guarantees the quiescence of the prepubertal gonad; the second is activated at the termination of juvenile development and leads to the pubertal resurgence in pulsatile GnRH release that results in stimulation of the pituitary-gonadal axis culminating in gonadarche, the major physiological process underlying primate puberty (Plant and Witchel, 2006).

The foregoing “on-off-on” pattern of hypothalamic GnRH pulse generator activity that is characteristic of postnatal development in primates is largely independent of the gonad. This was first demonstrated by Conte et al. (1975) who found that circulating gonadotropin levels in children with gonadal dysgenesis were elevated during infancy and at ages when puberty would have been anticipated in gonadally intact subjects but were low during the intervening childhood years. This pattern in gonadotropin secretion throughout postnatal development in primates was called diphase (Conte et al, 1975) and is exemplified by the gonadal male rhesus monkey (Plant, 1985; Figure 1).

While the switch that results in the pubertal resurgence of pulsatile GnRH release has been studied more extensively than that which suppresses the GnRH pulse generator in late infancy, in neither situation is the underlying neurobiology well understood. In both cases, however, it is reasonable to propose that the marked developmental changes in GnRH pulsatility are associated with structural remodeling of the GnRH pulse generator and/or with molecular changes in the hypothalamus. In the case of structural plasticity, PSA-NCAM, a marker of neuronal plasticity (Sunshine et al., 1987; Theodosis et al., 1991), is expressed in the MBH of prepubertal monkeys (Perera et al., 1993), and a decrease in synaptic input to GnRH perikarya in the MBH has been reported between the juvenile and adult stages of development in the male monkey (Perera and Plant, 1997).

Thyroid Hormone (TH) and Brain Development

The thyroid gland under the stimulation of thyroid stimulating hormone (TSH) produces two hormones, thyroxine (T_4) and triiodothyronine (T_3) (Kopp, 2005). The vast majority of the TH secreted by the thyroid gland is T_4 ; T_3 is produced primarily from deiodination of T_4 in target tissue. T_3 is the most biologically active form of the hormone and tissue and circulating levels of T_3 are regulated, in part, by two deiodinases (D1 and D2) (Bianco and Larsen, 2005). TH (both T_4 and T_3) secretion in humans begins at ~four months of gestation and progressively rises until parturition (Fisher and Polk, 1989; Thorpe-Beeston et al., 1992; Porterfield and Hendrich, 1993). At birth there is a surge of TSH secretion initiating a rise in T_4 production and release. Circulating T_3 levels increase more gradually as liver D1 is being induced and more of the T_4 is converted to T_3 (Fisher and Polk, 1989). Circulating TH levels then decrease with age in children (Fisher et al., 1977; Parra et al., 1980). In like fashion, total plasma T_4 levels in the rhesus monkey are very high (10-13 $\mu\text{g/dl}$) during the first week of postnatal life, but decrease rapidly over the next two months, and a decelerated decline continues throughout the juvenile and late prepubertal periods (Mann et al., 2002).

TH plays an important role in normal brain development and differentiation, particularly late brain development, which in humans extends from the third trimester of gestation through the first 3 months of postnatal life (Fisher and Brown, 2000; Anderson, 2001). Late brain development is characterized by neuronal cell migration, synaptogenesis, myelination and proliferation including that of glia. Neuron proliferation occurs during the second trimester of gestation while differentiation, outgrowth of neurites and myelination extend from the third trimester through the first two to three years of postnatal life (Porterfield and Hendrich, 1993; Xue-Li et al., 1994). These latter processes, which are associated with increased brain protein content and weight, are regulated by TH either directly or indirectly (Anderson,

2001). The most critical window of TH-dependent brain development occurs during the first six to eight months of postnatal life in humans, although TH-dependent central nervous system (CNS) maturation extends postpartum for two to three years (Fisher and Brown, 2000).

Untreated human infants with congenital hypothyroidism, born to euthyroid mothers, where cross-placental movement of TH protects the fetal brain *in utero* (Vulsma et al., 1989; Calvo et al., 1990) exhibit a progressive cognitive loss and neurological impairment (Smith et al., 1957), that may be prevented by TH replacement (Derksen-Lubsen and Verkerk, 1996). More recent clinical data from patients with congenital defects in the MCT8 transporter further documents the importance of TH for normal brain development. This transporter which will be discussed in more detail later is essential for moving T_3 (the active form of TH) from its site of production in astrocytes into neurons of brain regions that are particularly sensitive to TH (Bernal, 2005; Visser et al., 2007). Patients with genetic defects in the MCT8 transporter (a condition known as Allan-Herndon-Dudley syndrome) have severe neurological deficits that include but are not limited to mental retardation, hypotonia, impairment or absence of speech, and delayed milestones of development (Dumitrescu et al., 2004; Friesema et al., 2004; Schwartz et al., 2005; Jansen et al., 2007; Schwartz and Stevenson, 2007; Friesema, Visser and Visser, 2010). Despite the severe TH-dependent impairment these patients exhibit elevated circulating free T_3 and lower T_4 concentrations which are indicative of abnormal TH transport. In sharp contrast to the severe permanent TH-dependent impairments of CNS development in patients with congenital hypothyroidism or Allan-Herndon-Dudley syndrome, children that acquire hypothyroidism between three years of age and adolescence do not suffer any permanent impairment of CNS function, but do exhibit reduced intellectual performance and growth retardation (Foley, Malvaux, and Blizzard, 1994).

The foregoing considerations have led us to use a TH deficiency in an attempt to block any structural remodeling in the hypothalamus that is associated with the activation of the two switches that govern the pattern of pulsatile GnRH release during postnatal primate development. We employed the male monkey because the brake imposed on pulsatile GnRH release during prepubertal development in this sex is more marked than that in the female (Figure 1) and we reasoned therefore that the effects of blocking TH-dependent neuronal plasticity would be more marked and easier to identify for the first time. Agonadal animals were used for the following reasons. First, the hiatus in pulsatile GnRH release during juvenile development in primates occurs independently of the testis, and castration greatly amplifies the initiation and termination of this hiatus in neuroendocrine activity (Plant, 1985, and see Fig 1) enhancing the reliability of using circulating LH to indirectly monitor developmental changes in pulsatile GnRH release (Plant, 1986). Second, gonadectomy eliminates any potential direct action of TH on testicular steroid secretion and therefore eliminates any confounding action of testicular feedback on the developmental pattern of pulsatile GnRH release.

Impact of hypothyroidism during infancy on the postnatal pattern of GnRH release

In order to create a TH deficit during infancy, agonadal male monkeys were thyroidectomized (Tx) within five days after birth (Plant et al., 2008). Within a week of Tx, plasma T_4 declined to undetectable levels and by 6 to 8 weeks of age signs of hypothyroidism were evident. Hypothyroidism during infancy, however, failed to prevent the arrest of GnRH pulse generator activity during the infant-juvenile transition (Figure 2) indicating that either plasticity in the hypothalamus during this phase of development is not obligatory for the “turn off” in pulsatile GnRH release or that any hypothalamic remodeling at this stage of development is TH independent. In two of the neonatally Tx monkeys, T_4 replacement was initiated at 19 weeks of age to reestablish a euthyroid condition during

juvenile development (Figure 2). This allowed for the assessment of the impact of a transient hypothyroidism during infancy on the timing of the pubertal resurgence of pulsatile GnRH secretion. The TH deficiency during infancy also failed to influence the timing of the pubertal resurgence of gonadotropin secretion (Figure 2). Thus, it appears that the remodeling of the neurobiological mechanisms that govern the pubertal reactivation of pulsatile GnRH release was not altered by a transient, albeit severe, TH deficit during infancy. It is to be noted here that while body weight exhibited complete catch-up following the initiation of T₄ replacement in the Tx animals, crown-rump length and bone age did not - resulting in a dissociation between the timing of the pubertal resurgence of GnRH secretion and skeletal development (Plant et al., 2008).

Impact of hypothyroidism during juvenile development on pubertal resurgence of GnRH release

A TH deficit in juvenile gonadal monkeys (15-19 mo of age) was created by oral treatment with the antithyroid drug, methimazole (MMI; 0.025 % in drinking water) described by Mann et al. (2006). Within eight weeks of initiating treatment, plasma T₄ concentrations had fallen to near undetectable levels. This severe hypothyroid condition delayed the pubertal rise in LH secretion by about 8 mo (Figure 3). Although we cannot exclude the possibility that an attenuated pituitary GnRH sensitivity may have contributed to the pubertal delay in LH secretion in the hypothyroid animals, we favor a predominantly hypothalamic effect on the GnRH pulse generator for the following reasons. In ovariectomized ewes, Tx prevents the decrease in GnRH and LH pulse frequency associated with increasing photoperiod as animals enter the non-breeding season (Moenter et al., 1991; Webster et al., 1991; Anderson et al., 2002), while pituitary response to GnRH shows little or no change with season (Jenkin et al., 1977; Brewer et al., 1995). In addition, while Tx in the adult rat was associated with an increase in the serum LH response to castration and an elevation in both the nadir and peak of LH pulses, the absence or the presence of TH (in excess) did not alter the synthesis, secondary structure or clearance of the gonadotropin (Freeman et al., 1975).

The TH deficiency in gonadal juvenile monkeys was associated with a severe retardation of somatic development including that of body weight gain, linear growth, and bone maturation (Mann et al., 2006). Although nutritional status is known to influence growth and the timing of puberty (Plant and Witchel, 2006), the retarded somatic development observed in the hypothyroid animals was unlikely to be the result of a chronic state of reduced energy intake because body mass indexes and circulating leptin levels (two indices of body fat and energy stores) indicated that the TH deficit did not result in a chronic energy deficiency. In fact, both of these markers of energy stores were actually elevated in hypothyroid animals compared with controls, strongly suggesting that the delayed pubertal resurgence of LH secretion was not a consequence of a nutritional deficit.

Whereas, in control monkeys the pubertal rise in LH secretion occurred within a very narrow age range (29.3 ± 0.2 months), only half of the hypothyroid animals exhibited reactivation of GnRH pulse generator activity by 36 months of age. In the remaining monkeys, the pubertal resurgence of pulsatile GnRH release was observed after the initiation of T₄ replacement at 36 months of age. As was to be anticipated, catch up growth was observed after replacement therapy was initiated. The observation that the pubertal LH rise occurred in 50% of the animals while they were profoundly hypothyroid suggests that the role of TH in initiating this developmental event is permissive rather than obligatory. This permissive effect of TH on the pubertal reemergence of GnRH pulse generator activity may result from a direct action of TH on the hypothalamus or from an indirect action of TH on somatic development.

Primary hypothyroidism in children is predominantly associated with a delay in puberty (Styne and Grumbach, 2008) that can be corrected with adequate T₄ replacement (Longcope, 2000). However, on rare occasions sexual precocity has been reported (Larsen and Ingbar, 1992; Jannini et al., 1995; Longcope, 2000). Enlarged testes or macroorchidism occurs in about 80% of boys with juvenile hypothyroidism (Styne and Grumbach, 2008), but circulating testosterone concentrations remain prepubertal, axillary and pubic hair fail to develop and Leydig cell maturation is absent (Laron et al., 1970; Kugler and Huseman, 1983; Castro-Magana et al., 1988; Hoffman et al., 1991; Longcope, 2000) suggesting that the hypothalamic - pituitary - testicular axis has not activated. The enlargement of the testes occurs as a consequence of an increase in the size of the seminiferous tubules that results from an increased number of Sertoli cells (Styne and Grumbach, 2008). The exact reason for the enhanced Sertoli cell proliferation has not been fully resolved. It was first proposed that the incomplete sexual development may result from overlapping negative feedback control in hypothyroidism resulting in increased secretion of both TSH and gonadotropin, and with the advent of sensitive radioimmunoassays it was later shown that both immunoactive and bioactive FSH, but not LH, were elevated in this syndrome and this monotropic rise could account for the enlargement of the testes (Pringle et al., 1988; Buchanan et al., 1988; Castro-Magana et al., 1988; Bruder et al., 1995). Alternatively, it has been suggested that either the high levels of TSH characteristic of untreated hypothyroid patients may lead to an FSH-like effect of TSH on Sertoli cell multiplication (Anasti et al., 1995), or the hypothyroid condition itself inhibits Sertoli cell differentiation thereby prolonging proliferation of this testicular cell type (Jannini et al., 1995). Much remains to be resolved concerning the role of TH status on pubertal development in the primate since clinical studies such as these with gonadally intact paradigms make it difficult to establish whether any perturbation to puberty is due to a central (hypothalamic) effect of TH on GnRH release or to a direct action on the gonad.

Site of the facilitative action of TH on the pubertal resurgence of pulsatile GnRH release

The site of the facilitative action of TH to permit the pubertal reactivation of pulsatile GnRH release must reside either at the level of the brain to directly interface with the hypothalamic GnRH pulse generator or at a peripheral (somatic) site or sites, which in turn communicate with the hypothalamus via a circulating endocrine signal that conveys information on TH-dependent somatic development. In regard to the latter, it has long been hypothesized that the achievement of a particular level of somatic development (e.g., body weight or fat/lean body mass and/or skeletal development) was essential to allow pubertal development to proceed (Frisch and Revelle 1970; Plant et al., 1989).

In order to begin to unravel this mystery, we have attempted to create a model of selective central hypothyroidism in the agonadal juvenile male monkey and then examine the consequences of this endocrine deficit on the timing of the pubertal reactivation of GnRH secretion. We reasoned that if the action of TH on this critical developmental event is indirect then a selective central hypothyroid state, in the face of a systemic euthyroid state, should not delay the pubertal resurgence in LH release as was observed earlier in animals rendered globally hypothyroid by treatment with MMI.

The approach to achieve a selective central hypothyroidism was based on a generally accepted concept that the major portion of biologically active TH (i.e., T₃) in the brain is produced *in situ* from the deiodination of T₄ after transport of T₄ from the circulation to brain glial cells that express D2 and conversion of T₄ to T₃ (Crantz et al., 1982; Bernal, 2005; Heuer et al., 2005). While it was once considered that TH, being lipophilic in nature, could readily enter cells passively by passing through the lipid bilayer of the cell membrane, evidence developed over the last decade has shown that a group of high affinity transporters are involved in facilitating the movement of these hormones into target cells (Hennemann et

al., 2001; Abe et al., 2002; Bernal, 2005; Heuer et al., 2005). Among these are two families of transporters, the organo anion-transporting polypeptides (OATP) and monocarboxylate anion transporters (MCT). The human and rat OATP (OATP-F and Oatp-14, respectively) transporters are preferentially expressed in brain tissue, and at least the Oatp-14 appears to be localized in the border of brain capillary endothelial cells as well as in the choroid plexus where it appears to be involved in moving T_4 across both the blood-brain barrier and blood-cerebrospinal fluid barrier, respectively (Bernal, 2005). Both the human and rat transporter more efficiently transport T_4 and reverse T_3 than T_3 . The MCT family of transporters is expressed in a variety of tissues including brain (Heuer et al., 2005). One of the members of this family (MCT8) is highly expressed in neurons of a large number of brain regions, particularly those that are sensitive to TH (Bernal, 2005) and has a slightly higher affinity for T_3 than T_4 (Friesema et al., 2003). It is thought that expression of MCT8 in these brain areas is involved in uptake of T_3 from its site of production in astrocytes (Bernal 2005). Tanycytes that line the third ventricle also express MCT8 and interestingly, D2, (Guadano-Ferraz et al., 1997; Tu et al., 1997), and processes from these cells extend into adjacent hypothalamic regions and the median eminence (Bernal, 2005); areas that are known to be involved in the regulation of GnRH secretion (Prevot et al., 2010). Theoretically, T_3 generated by tanycytes from T_4 from the cerebral spinal fluid could play a role in altering the interaction between cells and GnRH neurons. However, the preferred route for distribution of TH to the brain is through the blood-brain barrier rather than the blood-cerebral spinal fluid barrier and so the relative importance of T_3 generated by tanycytes in the processes by which TH regulates brain development remains an open question.

In the rat, greater than 80% of T_3 present in the brain is produced *in situ*. Since T_3 is less efficiently transported across the blood-brain barrier by Oatp14, in hypothyroid rats it is not possible to maintain normal brain levels of TH with systemic administration of T_3 (Escobar-Morreale et al., 1997). We deduced from the rodent data that it might therefore be possible to induce a selective central hypothyroid state in MMI-treated monkeys while maintaining normal metabolism in peripheral tissues and somatic development with systemic T_3 replacement.

To this end, three juvenile gonadal male monkeys (17 mo of age) were placed on oral MMI at a dose used in the earlier study to induce a profound hypothyroidism (Mann et al., 2006). Animals were immediately placed on T_3 replacement therapy (0.75 to 3 $\mu\text{g/kgBW/day}$) using sc implanted osmotic minipumps. As expected, plasma T_4 decreased to undetectable levels and the T_3 dose was adjusted to maintain peripheral euthyroid status and normal somatic development as reflected by body temperature, heart rate, linear growth and bone maturation. Plasma LH levels were monitored weekly to assess developmental changes in the activity of the GnRH pulse generator. Results were compared to historical data for globally hypothyroid (MMI-treated, no TH replacement) and for euthyroid monkeys from our laboratory (see above). The highest replacement dose of T_3 generated plasma T_3 concentrations that were greater than euthyroid levels, but which maintained heart rate and body temperature in the euthyroid range indicating that systemic thyroid status was relatively normal. Plasma TSH levels rose at the start of MMI treatment and then declined as the T_3 dose was adjusted but remained well above euthyroid levels. T_3 treatment supported progressive linear growth and bone maturation. Interestingly, the pubertal resurgence of LH secretion in these animals was not delayed, as was the case in the earlier study of globally hypothyroid monkeys (see above).

The T_3 replaced MMI-treated monkeys were killed at 32 mo of age and brain (cortex and MBH) and peripheral tissues including liver were harvested to measure the activity of D2 and D1 as an indication of central and peripheral TH status, respectively, using assays previously described (Balzano et al., 1990; Dumitrescu et al., 2006). Levels of activity of D2

in the cerebral cortex and D1 in the liver suggested that while peripheral T₃ administration to the MMI-treated monkeys was sufficient to maintain a peripheral euthyroid condition (no differences in liver D1 activity compared to thyroid intact castrate controls), it was not sufficient to fully correct the central hypothyroidism since D2 activity in the cortex was higher (after log transformation of the data, $P=0.0349$) in the T₃-treated MMI animals than controls. (Table 1). D2 activity was not detected in the MBH of either group. Before the results of this pilot study of the rhesus monkey can be interpreted with confidence, the study of additional animals is required. In particular, it will be necessary to examine D2 activity in brain and D1 activity in liver of globally hypothyroid monkeys in order to fully appreciate the degree, and therefore the significance, of the central hypothyroidism achieved in the MMI-treated T₃ replaced animals. In this regard, the impact of mutations in MCT8 on pubertal development in boys merits comment (Schwartz and Stevenson, 2007; Wemeau et al., 2008). This is because, conceptually, both the patients with mutations of this transporter and the MMI-treated monkeys replaced with T₃ represent models of relative central hypothyroidism. Consistent with the results of the monkey study, boys with mutations in MCT8 are reported to generally undergo puberty at the appropriate time (Schwartz and Stevenson, 2007). When puberty has been delayed in these “experiments of nature”, malnutrition and extremely low body weight have also been reported (Wemeau et al., 2008). The importance of MCT8 for the maintenance of normal CNS levels of TH and brain development, however, was underlined by the fact that circulating levels of free T₃ were substantially above normal in these patients (Schwartz and Stevenson, 2007; Wemeau et al., 2008).

The role of the medial basal hypothalamus (MBH)—Regardless of the relative importance of direct (CNS) versus indirect (peripheral/somatic) actions of TH in regulating the developmental pattern of pulsatile GnRH release, it seems reasonable to propose that the MBH-pituitary unit will be the site of TH signaling in the case of the first possibility as well as the site at which the putative TH-dependent circulating somatic cues signal to the GnRH pulse generator in the case of the second possibility. This proposal is based on the finding that surgical isolation of the MBH in prepubertal female rhesus monkeys does not influence the time of the onset of puberty suggesting that the central mechanisms that govern the postnatal developmental pattern of pulsatile GnRH release in the primate reside within in the MBH-pituitary unit (Norman et al., 1981; Krey et al., 1981).

A considerable body of compelling evidence indicates that the MBH is a major site of TH action in regulating annual cycles in the activity of the GnRH neuronal network in seasonally breeding species (see Ebling, 2010). Elegant studies in quail (long day breeder) and sheep (short day breeder) have recently demonstrated that long days increase TH availability in the basal hypothalamus (Yoshimura et al., 2003; Hanon et al., 2008), and the resulting local increase in TH activity is posited to underlie the ability of the hypothalamic GnRH neuronal network to respond to changing photoperiod in a species specific appropriate manner (Ebling, 2010). Accordingly, elevated TH activity is associated with a suppression of the GnRH drive in short day breeders such as sheep, and with activation of GnRH release in long day breeders such as quail. An analogous mechanism is possible operative in the rhesus monkey: a short day seasonally breeding primate (Lancaster and Lee, 1965).

That puberty in seasonally breeding species may be viewed as recurring each year at the time of the seasonal activation of the reproductive axis has been expounded (Ebling and Foster, 1989; Ebling, 2010), but the relevance of this notion to understanding the mechanisms that time the onset of puberty in primates, where sexual maturation is delayed for several years (Plant and Witchel, 2006) is unclear. In the rhesus monkey, for example, the hypothalamus of a spring born feral male presumably receives photoperiodic input in the

fall of the first and second year of life (at ages 6 and 18 months), which in the postpubertal animal would allow for the seasonal recurring activation of the GnRH network to unfold. The GnRH network in the hypothalamus of the 6 and 18 month old juvenile monkey, however, is “asleep” as a result of a **developmental** brake and therefore the recurring seasonal input cannot be manifest. Removal of the developmental brake is typically initiated during the third year of life and leads to a reawakening of the GnRH pulse generator, which enables this neural network to be regulated for the first time by season. According to this model, the pubertal reactivation of GnRH release under feral conditions can only take place during the breeding season. If the developmental brake on the prepubertal GnRH pulse generator is removed between breeding seasons it will not be manifest because of the secondary seasonal inhibition that delays pubertal reactivation until the next breeding season. Extrapolating this model to boys where puberty is delayed for more than ten years helps to conceptualize the notion that, while developmental and seasonal regulation of GnRH pulse generator activity may be dependent on TH, they also represent distinct control systems. The mystery of primate puberty will not be unlocked until the mechanism responsible for lifting the developmental brake on GnRH release, that is imposed for several years during the juvenile phase of development, is elucidated. Lastly, the foregoing view of the timing of puberty in primates appears theoretically to be most compatible with the notion of a somatic site of TH action in timing this critical developmental event.

Within the primate MBH, the arcuate nucleus (or infundibular nucleus) – a periventricular structure at the base of the third cerebroventricle – is essential for maintaining the pulsatile GnRH drive that sustains gonadotropin secretion throughout adulthood (Plant et al., 1978). This hypothalamic nucleus in both monkey and man contains a population of neurons that express *KISS1*, the gene that encodes the neuropeptide, kisspeptin (Shahab et al 2005; Rometo et al 2007; Ramaswamy et al 2008), a major and potent stimulator of GnRH/LH release in primates and other species (Shahab et al 2005; Plant et al., 2006; Oakley et al., 2009; Jayasena et al., 2009). The kisspeptin receptor (KISS1R, also called GPR54) is expressed by GnRH neurons (Oakley et al., 2009) and in the monkey local administration of a KISS1R antagonist in the median eminence suppresses GnRH release (Roseweir et al, 2009). Most recently, it has been demonstrated that, in the monkey as in non-primate species, kisspeptin neurons of the arcuate nucleus also express neurokinin B (Ramaswamy et al 2010), that is encoded by *TAC* (Almeida et al, 2004) and which also stimulates GnRH release in the monkey (Ramaswamy et al 2010). Because these arcuate neurons also express dynorphin (Goodman et al., 2007; Rometo and Rance, 2008), Cheng et al. (2010), have coined the acronym, KNDy, to describe their phenotype. Mutations within these two signaling pathways lead to hypogonadotropic hypogonadism and delayed or absent puberty in man (deRoux et al., 2003; Seminara et al., 2003; Topaloglu et al., 2009). Moreover, at the time of puberty in the monkey *KISS1* expression is upregulated in the arcuate nucleus (Shahab et al 2005) and kisspeptin release in the median eminence is increased (Keen et al., 2008). For these reasons it seems reasonable to propose that the arcuate nucleus is an attractive candidate for the site of action for either TH itself (direct) or of any circulating cue that relays information of somatic development to the brain (indirect).

While TH receptors have not been specifically identified in KNDy neurons, the four isoforms of this receptor (α_1 , α_2 , β_1 and β_2) are found in the infundibular (arcuate) nucleus of the human hypothalamus, as well as in other hypothalamic nuclei including the paraventricular, and supraoptic nuclei (Alkemade et al., 2005). Similarly in the rodent brain, TH receptors are highly expressed in the hypothalamus (Bradley et al., 1989; Sasaki et al., 1991; Childs et al., 1991; Yen et al., 1992; Li and Boyages, 1996). TH receptors (α isoforms) have also been found colocalized with GnRH neurons in the hypothalamus of sheep and hamsters (Jansen et al., 1997). Since significant numbers of GnRH cell bodies are localized in the MBH of primates (Silverman et al., 1994), the GnRH neuronal network is

therefore also a potential site for any direct actions of TH underlying the permissive role of this hormone in triggering puberty. In the case of an indirect action of TH there would be no need to posit the necessity of hypothalamic TH receptors.

While an adenohypophyseal site of action of TH or of TH dependent circulating cues of somatic development has not been empirically addressed, comparative considerations discussed above argue against the pituitary playing a major role in the mediating TH action on the neuroendocrine axis governing reproduction.

Summary

The pubertal resurgence of pulsatile GnRH release in male monkeys, which triggers the onset of puberty in this and other species of primate, is dependent on a permissive action of TH at this stage of development. Interestingly, TH action during infancy does not appear to be involved in either initiating the restraint that is imposed on pulsatile GnRH release during the infantile-juvenile transition or the pubertal reactivation of the GnRH pulse generator. The delay in the pubertal reactivation of the GnRH pulse generator that is induced by juvenile hypothyroidism is linked with retardation of somatic development, and this association has confounded our ability to define the mechanism of action of TH on the timing of this critical developmental event. A number of questions remain unresolved: Is the permissive action of TH required for the onset of puberty a direct consequence of TH signaling on hypothalamic centers regulating GnRH secretion, or is it an indirect consequence of TH action on somatic tissues that is relayed to the hypothalamus by circulating cues that reflects TH-dependent somatic maturation? Regardless of whether the action of TH in permitting puberty to occur is direct or indirect, we hypothesize that the hypothalamic locus of this control system is likely to be the arcuate (infundibular) nucleus within the MBH, and that KNDy neurons in this nucleus probably represent a major link relaying TH action to the GnRH neuronal network (Figure 4).

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Abbreviations

GnRH	gonadotropin releasing hormone
PSA-NCAM	embryonic neuronal cell adhesion molecule
MBH	medial basal hypothalamus
TSH	thyroid stimulating hormone
TH	thyroid hormone
T₄	thyroxine
T₃	triiodothyronine
D1	type 1 deiodinase
D2	type 2 deiodinase

CNS	central nervous system
Tx	thyroidectomized
MMI	methimazole
FSH	follicle stimulating hormone
LH	luteinizing hormone
OATP	organic anion-transporting polypeptide
MCT	monocarboxylate anion transporter
MBH	mediobasal hypothalamus

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Figure 1.

The on-off-on pattern of gonadotropin-releasing hormone release during postnatal development in agonadal male (stippled area) and female (closed data points $\pm$ error bars) rhesus monkeys, as reflected by circulating mean luteinizing hormone (LH) (top panel) and follicle-stimulating hormone (FSH) (bottom panel) concentrations from birth to 142-166 weeks of age. Note, in males, the intensity and duration of the prepubertal hiatus in the secretion of FSH, and LH to a lesser extent, is exaggerated in comparison to females. Adapted from TM Plant. Puberty in primates. In E. Knobil, JD Neill, eds. *The Physiology of Reproduction*, 2nd edn. New York: Raven Press Ltd, 1994: Vol 2, Chapter 42, 453-485, Copyright Elsevier, 1994.

Figure 2.

Relationship between circulating T_4 levels (top panel) and the time-course of LH secretion (bottom panel) throughout postnatal development in agonadal monkeys that were either Tx at birth ($N=3$, two of these animals were replaced with TH at 19 weeks of age; open circles) or sham thx at birth ($N=3$, closed circles). Shaded areas represent the period of TH replacement in thx animals. Note the transient, albeit profound, hypothyroidism during infancy failed to interrupt either the "turn off" of GnRH pulse generator activity (as reflected by plasma LH concentrations) during infancy or the reactivation of this neuroendocrine system at the termination of the juvenile phase of development. Reproduced by modifying Figures 3 and 4 from Plant et al. (2008) (Copyright Blackwell Publishing Ltd).

Figure 3.

Time-course in plasma concentrations of T₄ (top panel) and LH (bottom panel) in control (solid circles) and MMI-treated (open circles) agonadal male rhesus monkeys from 15-42 months of age. The shade area represents the period of T₄ replacement in MMI-treated animals. Note the hypothyroid state was associated with a delay in the pubertal resurgence of pulsatile GnRH release as reflected by circulating LH concentrations. Reproduced by modifying Figures 1 and 2 from Mann et al. (2006) (Copyright Blackwell Publishing Ltd).

Figure 4.

Potential mechanisms for the permissive action of TH on the pubertal resurgence of pulsatile GnRH release in primates. TH may act 1) directly at the level of the hypothalamus to modulate stimulatory signaling by KNDy neurons in the arcuate nucleus to GnRH neuron (1a) and/or the activity of the GnRH neuronal network itself (1b), or 2) indirectly via an action on peripheral tissues that determines the rate of somatic maturation and is reflected by a circulating cue(s) (3) that is tracked by the hypothalamus. A role of other neuropeptides and neurotransmitters in dictating the postnatal pattern of pulsatile GnRH release is not excluded. A direct action of TH at the level of the pituitary gonadotroph (4) to alter the response to GnRH has yet to be eliminated empirically.

Table 1

Effect of peripheral T₃ administration on D2 activity in the cerebral cortex and pituitary and D1 activity in the liver in monkeys in which T4 secretion had been chronically inhibited by MMI-treatment.

	D2 activity CTX (fmol/h/mg protein)	D2 activity pituitary (fmol/h/mg protein)	D1 activity liver (pmol/h/mg protein)
euthyroid controls	30.8 ± 6.5	1161.4 ± 177.3	32.6 ± 0.2
MMI + T ₃	86.4 ± 19.6 *	1323.6 ± 311.3	30.8 ± 1.8

* Significantly different at the P=0.0349 level from the euthyroid controls after log transformation of the raw data. The raw data did not quite reach levels of significance (P=0.0545).