Photoperiodic regulation of puberty in seasonal species

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ABSTRACT

Puberty occurs seasonally in the majority of mammals native to temperate or arctic latitudes, and in species with sufficiently long life spans puberty can be considered to reoccur on an annual basis. The precise timing of puberty and the annual reoccurrence of fertility reflects an interaction of changes in ambient daylength (photoperiod) and endogenous long-term timing processes, which in some species constitute circannual clocks. Recent studies reveal an unexpected common signalling pathway for photoperiodic information in mammals and birds: changes in secretory activity of the pars tuberalis in the pituitary stalk signal to the tanycyte cells in the ependyma lining the third ventricle. The target genes in the tanycytes encode the deiodinase enzymes that regulate the availability of thyroid hormone in the hypothalamus. Central availability of thyroid hormone appears to be the key determinant of seasonal reproductive transitions. Given the necessity of thyroid hormone for the initial development of the central nervous system, it is hypothesized that at puberty and seasonal reoccurrences of fertility it is the changing local levels of thyroid hormone that orchestrate hypothalamic plasticity, ultimately impinging upon the secretion of GnRH.

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1. Puberty as a seasonal event

Mammals have evolved a variety of strategies to ensure that offspring are born in the optimal season for survival and growth, which for most terrestrial species in temperate latitudes is the spring. In some species such as roe deer this arises principally from the neuroendocrine control of the timing of reactivation and implantation of the blastocyst, a process dependent upon changes in prolactin secretion. However, in the majority of species it is the timing of ovulation and mating and therefore conception that is regulated.

The follicular development in the ovary and testicular maturation which lead to these events are clearly driven by increased gonadotropin-releasing hormone (GnRH) and thus gonadotropin secretion, but for most species the attainment of fertility that defines puberty is not the initial activation of the hypothalamic-pituitary-gonadal axis. Earlier periods of activation clearly occur, during fetal development, for example in sheep (Mesiano et al., 1991), or in the perinatal and early neonatal period, for example in primates (Plant, 1986) and rodents (Ford and Ebling, 2000; Castellano et al., 2006). Thus while puberty is unique in the sense that it is the first occasion on which mature gametes are produced and the sexual behavioral repertoire is expressed, it is not unique in neurobiological terms, as in many seasonal species it is neither the first nor the last activation of the hypothalamo-pituitary-gonadal (H-P-G) axis. In species with a long life span the initial activation of GnRH synthesis and secretion occurs early in development, thus puberty should be considered as a reactivation of the H-P-G axis (Ebling, 2005). Moreover, in many seasonal species the H-P-G axis downregulates for part of the year, so essentially the annual onset of fertility is a reoccurrence of puberty. It is possible to identify certain mechanistic differences between puberty and subsequent seasonal onsets of fertility, for example there are absolute growth requirements for the former which may not be invoked later, but in terms of seasonal regulation of GnRH secretion there are likely to be common processes underlying puberty and subsequent seasonal onsets of fertility (Ebling and Foster, 1990; Lincoln and Short, 1980).

2. Transduction of photoperiodic information to the neuroendocrine axis

Although a wide variety of environmental parameters vary seasonally and can influence the timing of activation of the H-P-G axis, including changes in ambient temperature and food supply, unquestionably changes in daylength are the dominant cue. This is simply because as an unvarying geophysical cue from year to year it can be used to predict or anticipate the changing seasons, which is highly important for species with gestation periods of several months. For example, sheep have a 5 month gestation...
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must occur in the preceding fall. It is therefore the transition from
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must occur in the preceding fall. It is therefore the transition from
long summer photoperiods to shorter autumnal photoperiods that
provides the key time cue initiating puberty. The photoneuroen-
docrine circuitry which underlies this process is well established,
as illustrated in Fig. 1. Since the identification of melatonin in
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mals (Ebling and Barrett, 2008; Morgan and Hazlerigg, 2008). A
combination of ambient luminescence perceived by the retina and
circadian function of the suprachiasmatic nucleus drive melatonin production during the dark phase, such that the duration of melatonin secretion is a neurochemical representation of night length. In the last decade major advances have been made in understanding the mechanistic basis of three aspects of this pathway. First, the molecular basis of the circadian timing mechanism in suprachiasmatic nucleus neurons has become established, consisting of an overlapping series of transcriptional feedback loops (Hastings et al., 2007). Second, a population of ancestral retinal ganglion cells expressing melanopsin as a photopigment has been identified as the key photic input regulating the function of the suprachiasmatic nucleus (Gooley et al., 2001). Third, and the focus of this review, is the surprising discovery that the pars tuberalis of the pituitary gland is a key site of melatonin action, and the equally surprising discovery that signalling from the pars tuberalis back to the ependymal cell layer in the hypothalamus is a major pathway by which pubertal development/seasonal onsets of fertility and other seasonal physiological and behavioral traits are regulated.

3. Unexpected players: the pars tuberalis and tanycytes

Studies in sheep, rhesus monkeys and various hamster species utilizing pinealectomy and/or replacement of physiological concentrations and patterns of melatonin provide convincing evidence that melatonin really is the key hormone regulating the seasonal timing of puberty (Ebling and Foster, 1989; Bartness et al., 1993). However, identification of the sites of action of melatonin has proved to be elusive. Autoradiographic receptor binding studies using $^{125}$I-iodomelatonin as a radiolabelled ligand reveal fairly substantial differences in the distribution of melatonin binding across species where puberty is regulated seasonally. For example, in the sheep, melatonin binding is very widespread in the forebrain, including cortical areas, the hippocampus, medial preoptic area and anterior hypothalamus, tuberal hypothalamus and mamillary nuclei (Bittman and Weaver, 1990). In contrast, the distribution of binding is largely restricted to the suprachiasmatic nucleus and midline thalamic nuclei in the Siberian hamster (Duncan et al., 1989). A rather limited number of in situ hybridization studies provide some complementary evidence, but to date the immunohistochemical tools have been of very limited value in determining the precise cellular localization of the melatonin receptors encoded by the MT1 and MT2 genes (Williams et al., 2001). Inferences about the site of action of melatonin have been made on the basis of both microimplant studies and lesion studies. Thus, microimplants placed in the posterior hypothalamus and median eminence region of sheep are effective in regulating seasonal reproductive responses whereas those in more rostral regions are not (Malpaux et al., 1998). However interpretation of such studies is difficult because it cannot be precisely established how far physiologically relevant concentrations of melatonin might diffuse away from the implant site. Lesion studies carried out principally in rodents have not revealed with any consistency which hypothalamic regions are necessary for the actions of melatonin on the reproductive axis. For example lesions of the $^{125}$I-iodomelatonin in the mediobasal hypothalamus but not those in the suprachiasmatic nucleus block reproductive responses to melatonin in the Syrian hamster (Maywood and Hastings, 1995), whereas lesions of binding sites in the suprachiasmatic are reported to block the effects of melatonin in the Siberian hamster (Bartness et al., 1991). Interpretation of lesion studies is compromised by the difficult issue of whether such regions are necessary because they are actually mediating the effects of melatonin, or whether they are necessary because they provide a permissive input which allows the seasonal mechanisms to operate.

Fig. 1. A hypothetical model of the steps in the photoneuroendocrine pathway by which changes in daylength ultimately regulate GnRH secretion and puberty in seasonal species. Note that in this model the tanycytes are the key hypothalamic cell type transporting T4 into the brain via the MCT8 transporter, that TSHβ is a key signal produced by the pars tuberalis which regulates deiodinase gene expression, and that it is the relative activity of type II (DIO2) and type III deiodinase (DIO3) which determines the availability of T3.
The most consistent feature of these ligand binding studies is the identification of $^{125}$I-iodomelatonin binding in the pars tuberalis across all species studied (Morgan and Williams, 1996). This was initially viewed as a likely site of action of melatonin in regulating prolactin release because a) it had been demonstrated that seasonal rhythms in prolactin production persisted in male sheep where the pituitary had been disconnected from the hypothalamus (Lincoln and Clarke, 1994), and b) because in vitro studies demonstrated that an extract of pars tuberalis cells or tissue could stimulate prolactin release from cultures of lactotrophs derived from the anterior pituitary (Morgan et al., 1996). However the possibility that signals from the pars tuberalis could act back within the hypothalamus to regulate GnRH synthesis and release opposed the dogma instilled by Geoffrey Harris that releasing factors from the hypothalamus regulate GnRH synthesis and release opposed the dogma instilled by releasing factors from the hypothalamus.

The identification of this unexpected pathway has arisen from two lines of work. First, there is a long literature indicating a role for the thyroid gland in seasonal reproductive maturation. As reviewed by Bechtold and Loudon (Bechtold and Loudon, 2007), studies as far back as the 1930s demonstrated that removal of the thyroid gland disrupts the onset of seasonal fertility in European starlings and ducks. This work was rediscovered by Follett and colleagues, who demonstrated that appropriate replacement of thyroid hormone would restore seasonal reproductive transitions in starlings (Nicholls et al., 1984) and Japanese quail (Follett and Nicholls, 1985). Such observations were extended to sheep (Moenter et al., 1991; Webster et al., 1991; Parkinson and Follett, 1995), suggesting a commonality of mechanisms in birds and mammals, but the physiological relevance of these studies only became apparent when it was discovered that expression of the genes encoding the selenium-dependent deiodinase enzymes which regulate local availability of thyroid hormone in the brain are themselves regulated by photoperiod (Table 1). Although almost all neurons in the brain express the THα receptor, rather surprisingly expression of the monocarboxylase transporter MCT8 which facilitates thyroxine (T4) uptake from the circulation into the brain, and expression of type II deiodinase which catalyses the conversion of T4 to tri-iodothyronine (T3) occurs predominantly in hypothalamic tanycytes (Ceballos et al., 2009; Tu et al., 1997). Thus tanycytes are likely to play a key role in determining T4 uptake and T3 availability in the brain.

Across the species studied so far the photoperiodic regulation of deiodinase gene expression shows a consistency in that long-day photoperiods increase T3 availability whereas short-day photoperiods decrease this. However, there appear to be some species differences in the specific regulation of type II deiodinase (DIO2) which promotes conversion of T4 into the more bioactive T3, and type III deiodinase (DIO3) which promotes conversion of T4 into the inactive reverse T3 and catabolises T3 to T2. In most species including the quail (Yoshimura et al., 2003; Yasuo et al., 2005), sheep (Hanon et al., 2008), Syrian hamster (Revel et al., 2006) and photoperiodic strains of mice (Ono et al., 2008) and rats (Yasuo et al., 2007), DIO2 is upregulated by long days and downregulated by short days (Table 1), but this is not the case for seasonal transitions in fertility in the post pubertal Siberian hamster where it is short-day induced upregulation of DIO3 (Barrett et al., 2007) that is key (Table 1). Short-day regulation of DIO3 has also been observed in melatonin-competent strains of laboratory mice (Yasuo et al., 2009). The only species where this pattern is not clear is the Saanen goat where long days have been shown to downregulate DIO2 expression in the medial and posterior hypothalamus, however in the more rostral region of the ventral ependymal upregulation of DIO2 was observed. Regulation of DIO3 in the goat has not yet been reported, so it is possible that this is the key iodinase which regulates T3 availability in this species.

The second line of evidence which has led to the current understanding is that seasonal changes in the ultrastructure of the pars tuberalis (Wittkowski et al., 1984; Merks et al., 1993) and in TH production in this tissue (Wittkowski et al., 1988; Bockmann et al., 1996) had long been observed, but the functional significance of such changes was unknown. Close observation of the pars tuberalis revealed that tanycyte processes are in close apposition to some of these cells (Fig. 2), and in situ hybridization studies reveal that the ependymal cell layer containing the tanycyte cell soma expresses the gene encoding the TH receptor (Hanon et al., 2008). Functional evidence that this paracrine system consisting of cells in the pars tuberalis secreting TSHβ which acts upon TH receptors expressed in tanycyte processes comes from a variety of sources. In quail, a single long day is capable of initiating the stimulation of the reproductive axis, and induction of TSHβ gene expression in the pars tuberalis is one of the first events to be observed in this photoperiodic paradigm (Nakao et al., 2008). Correspondingly, intracerebroventricular treatment of quail in the short day prepupal condition with TSHβ can induce DIO2 expression and induce gonadal maturation, whereas immunoblockade of TSHβ prevents the induction of gonadal maturation in quail induced by long photoperiods (Nakao et al., 2008). A similar series of events occurs in mammals. Studies in the rat combining immunohistochemistry

### Table 1

<table>
<thead>
<tr>
<th>species</th>
<th>DIO2</th>
<th>DIO3</th>
<th>reference</th>
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<tbody>
<tr>
<td>quail</td>
<td>↑</td>
<td>↓</td>
<td>Yoshimura et al., 2003, Yasuo et al., 2005</td>
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<tr>
<td>Syrian hamster</td>
<td>↑</td>
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<td>Revel et al., 2006</td>
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<td>sheep</td>
<td>↑</td>
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<td>Hanon et al., 2008</td>
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<td>Saanen goat</td>
<td>↑↓↓</td>
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<td>Yasuo et al., 2006</td>
</tr>
<tr>
<td>CBA/N mouse</td>
<td>=</td>
<td>=</td>
<td>Ono et al., 2008</td>
</tr>
<tr>
<td>C3H mouse</td>
<td>↑</td>
<td>↓</td>
<td>Yasuo et al., 2009</td>
</tr>
<tr>
<td>Fischer 344 rat</td>
<td>↑</td>
<td>=</td>
<td>Yassuo et al., 2007</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>=</td>
<td>=</td>
<td>Revel et al., 2006, Yasuo et al., 2007</td>
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Cross-species comparison of photoperiodic regulation of gene expression for type II deiodinase (DIO2) and type III deiodinase (DIO3) in the ventral ependymal cell layer. 

a decreases DIO2 expression has been noted in juvenile Siberian hamsters exposed to SD (Watanabe et al., 2004).

b comparison made in goats exposed to natural daylength in March (‘SD’) compared to goats transferred to LD in November. Increased DIO2 expression was observed in the rostral arcuate region, but decreased expression occurred more caudally.

c melatonin-producing strain of mouse.

d Photoperiodic strain of rat.

e Non-photoperiodic strain of rat. ↑ indicates gene upregulated, ↓ indicates gene downregulated, n/a: no data available.
and autoradiography reveal that TSHβ-immunoreactive cells in the pars tuberalis bind melatonin, suggesting a direct action of melatonin on thyrotrophs in the pars tuberalis (Klosen et al., 2002). This is of functional significance because exposure of sheep to long photoperiods rapidly induces TSHβ gene expression in the pars tuberalis, and consequently upregulation of DIO2 in the ependymal cell layer (Hanon et al., 2008). The expression of the TSH receptor (TSH-R) has been clearly demonstrated in the ependymal cell layer of the sheep implicating a direct action of TSHβ on this cell layer, and infusions of TSHβ into the ventricular system of the sheep induces DIO2 gene expression (Hanon et al., 2008). Further evidence for the importance of TSHβ signalling comes from a recent study in mice where the suppression of DIO2 and induction of DIO3 expression which can be achieved by melatonin treatment in melatonin-deficient C57BL/6J mice is blocked in TSHR-null mice on the same genetic background (Ono et al., 2008).

Thus the molecular events underlying photoperiodic signal transduction appear to be remarkably similar in birds and mammals, with the important difference that the induction of TSHβ gene expression in the pars tuberalis reflects the changing duration of melatonin in mammals (Fig. 1), whereas birds do not use the retinal-pineal-melatonin for photoperiodic time measurement, but rely on extraretinal photoreceptors (Foster and Soni, 1998). However, increases T3 availability is not in itself a stimulatory signal to the GnRH secretory system, as in long-day breeders such as the quail and Siberian hamster, increased T3 availability is associated with initiation of reproductive activity, whereas in short-day breeders such as the Soay ram it is decreased T3 availability which is associated with seasonal puberty.

4. Functional evidence for a key role for thyroid hormone

Despite the substantial body of studies reporting consistent effects of photoperiod on DIO2 and DIO3 expression, only a small proportion of the studies in quail and goats have confirmed that this change in gene expression is associated with a change in hypothalamic T3 concentrations (Yoshimura et al., 2003; Yasuo et al., 2006). This may reflect a technical issue that in rodent brains the total amount of thyroid hormone in the hypothalamus is below the limit of sensitivity of many assays. In the quail, long-day exposure resulted in significant increases in both T4 and T3 content of the mediobasal hypothalamus (Yoshimura et al., 2003), the latter being consistent with the upregulation of DIO2 and down-regulation of DIO3 in the quail in long photoperiods (Table 1). In contrast, in the long-day reproductively inactive state in the goat when DIO2 expression was low in the ependymal cell layer and tubero-infundibular recess, T3 levels were undetectable, whereas in the control (natural photoperiod) state when the goats had active testes, T3 levels were measurable (Yasuo et al., 2006). T4 levels did not vary in the two states, consistent with the view that T3 availability is the endocrine variable that is regulated. The difficulty with this study is that the goat is the only species studied so far where long photoperiods results in a reduction in DIO2 gene expression (see Table 1), though it should be noted that in more rostral regions there was a LD-induced increase in DIO2 expression. More sensitive determinations of intrahypothalamic T3 levels in different photoperiodic states are clearly an important priority.

There is robust evidence from both sheep and Siberian hamsters that local manipulation of thyroid hormone levels in the hypothalamus produces functional effects consistent with the proposed photoneuroendocrine model (Fig. 1). As noted previously, female sheep that are thyroidectomized in the short-day induced breeding season do not spontaneously return to the anestrus condition unless thyroid hormone is replaced (Webster et al., 1991). Using this model, Viguie et al demonstrated that intracerebroventricular infusions of low concentrations of T4 would restore the transition to anestrus (Viguie et al., 1999), and likewise microimplants of T4 placed in the premammillary area or ventromedial preoptic area have the same effect (Anderson et al., 2003). It is assumed that either the local concentrations of T4 are sufficiently high to ensure a degree of bioactivity, or that sufficient DIO2 activity is present to catalyze conversion to T3. In this model, the increased thyroid hormone availability promotes the progression from a reproductively active to an inactive state. This may seem initially paradoxical, but the sheep is a short day breeder so this is an appropriate ‘long day’ response. A more holistic view is that the function of changing thyroid hormone availability is to transduce photoperiodic information which engenders a change in state of the hypothalamus,
rather than being a specific drive to a particular neuroendocrine axis (Barrett et al., 2007).

5. Central actions of thyroid hormone

Perhaps the major limitation of the current model is that the mechanisms of action of T3 and the nature of the changing state of the hypothalamus are largely conjectural. One might envisage a number of processes that T3 regulates which result in pubertal/seasonal activation of GnRH secretion (Fig. 1). First, a substantial proportion of GnRH neurons coexpress the thyroid hormone receptor (TRH) receptor in the sheep and Syrian hamster (Jansen et al., 1997). However, it seems unlikely that a nuclear receptor would directly determine the changes in pulsatile secretion of GnRH that provide the ultimate drive to the reproductive axis. There is evidence that the end feet of tanycyte processes surround the terminals of GnRH axons in the median eminence, and that changes in this glial ensheathment contribute to different secretory patterns (Rodriguez et al., 2005; Prevot et al., 1999; Yamamura et al., 2004). Since DIO2 is likely to be expressed in these same tanycytes the idea of a direct effect of T3 within the cells producing it is attractive, and there is experimental evidence that microimplants of T3 placed in the mediobasal hypothalamus of quail maintained in short days will reduce glial ensheathment of GnRH terminals, and this is associated with testicular growth (Yamamura et al., 2006).

However, the observation that puberty is associated with increased DIO2 expression/T3 availability in species which undergo puberty in short days (sheep) is consistent with the idea of a direct effect of T3 within the cells producing it. Further support for this comes from the elegant studies of Xu et al. (2005): the case report of delayed puberty in a boy with a congenital MCT8/H9251 receptor in the sheep and Syrian hamster (Jansen et al., 1998). The dependence of seasonal changes in neurogenesis have long been noted in brain centers associated with song production in birds (Nottebohm, 2004). There have also been meticulous ultrastructural studies providing evidence for changes in synapse density on GnRH neurons associated with seasonal changes in fertility in female sheep (Lehan et al., 1984; Adams et al., 2006). Given the relatively gradual progression of the neuroendocrine processes causing puberty, the notion that there may be a series of discrete events by thyroid-hormone-dependent changes in gene expression which ultimately change the growth factor milieu of the hypothalamus and plasticity in neural inputs to GnRH and tanycytes is very attractive, and worthy of further study.

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One study conducted in the Syrian hamster has provided evidence for a seasonal difference in the rate of neurogenesis in the hippocampus, as inferred from the differential uptake of BrdU in cells in the dentate gyrus (Huang et al., 1998), and seasonal changes in neurogenesis have long been noted in brain centers associated with song production in birds (Nottebohm, 2004). There have also been meticulous ultrastructural studies providing evidence for changes in synapse density on GnRH neurons associated with seasonal changes in fertility in female sheep (Lehan et al., 1984; Adams et al., 2006). Given the relatively gradual progression of the neuroendocrine processes causing puberty, the notion that there may be a series of discrete events by thyroid-hormone-dependent changes in gene expression which ultimately change the growth factor milieu of the hypothalamus and plasticity in neural inputs to GnRH and tanycytes is very attractive, and worthy of further study.
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