Hypothalamic Thyroid Hormones: Mediators of Seasonal Physiology

Over the past decade, the field of biological timing has witnessed some remarkable discoveries involving the molecular mechanisms that underlie the endogenous circadian clock. In most vertebrates, one crucial output from that clock is the rhythmic, nightly production of the pineal indoleamine hormone, melatonin. Melatonin rhythms are regulated by outflow from the master circadian clock in the hypothalamus, the suprachiasmatic nucleus (SCN), which in turn receives retinal input from the eyes. In mammals, melatonin provides a crucial hormonal signal controlling seasonal physiology and drives changes in multiple neuroendocrine pathways (see Fig. 1). Activity of the melatonin-secreting pathway in the pineal is exquisitely sensitive to light and, as a result, annual changes in the length of the day sculpt the melatonin profile to provide neuroendocrine systems with an accurate daily report of external photoperiod and, hence, seasonal time. Physiology as diverse as reproduction, seasonal fat cycles, growth and moult of the pelage, and hibernation are all regulated by melatonin and early studies, using daily melatonin replacement regimes, have demonstrated the primary role of this axis in mammals.

Remarkably, we have begun only recently to understand how the seasonal melatonin signal may be interpreted by the neuroendocrine axes. However, it has been clear for over 50 years that seasonal birds and mammals engage another hormone system in the regulation of seasonal response, that involving the thyroid hormones. Pioneering works by Benoit in ducks and by Woitkewitsch in starlings (1, 2) showed that removal of the thyroid gland dramatically altered the seasonal changes in gonadal maturity in these birds. These studies were rediscovered by Follett and Nicholls (3), among others, who showed that thyroidectomy blocked many of the seasonal responses to photoperiod in the Japanese quail, but that these could be restored by a single injection of T_4. Availability of the biologically active form of thyroid hormone, T_3, within the hypothalamus has now been linked to annual rhythms in several photoperiodic species, including birds, sheep, and hamsters (4, 5). The Siberian hamster, in particular, has proved to be an excellent model organism for studying the mechanisms underpinning seasonal physiology; it is easy to keep in laboratory conditions, and by simply changing the photoperiod from long, summer-like (14 h or more of light per day) to short, winter-like (10 h or less of light per day) photoperiods, a dramatic reprogramming of physiology occurs over the subsequent 3-month period, with gradual regression of the gonads to an infertile state, growth of a white winter pelage, and impressive reductions in body weight.

In this issue of *Endocrinology*, Barrett *et al*. (6) have used small micro-implants of T_3 applied locally within the hypothalamus and then exposed the hamsters to short photoperiods. T_3 prevented the onset of short-day physiology: T_3-treated animals did not exhibit the pronounced weight loss, decline in adiposity, or testicular regression normally associated with the transition to this photoperiod. Intriguingly, another axis, that regulating seasonal coat and hair growth was relatively unaffected. The availability of T_3 in the hypothalamus across both mammalian and avian species is greatly dependent on the local expression of two 5′-iodothyronine deiodinase enzymes: type-2 deiodinase (Dio2) and type-3 deiodinase (Dio3) (7). Dio2 catalyzes the formation of T_3 from its less potent precursor T_4, by outer ring deiodination, whereas Dio3 converts T_4 to reverseT_3, and T_3 to $3',3'$-T_2 by inner ring deiodination. It is thought that more than 75% of nuclear T_3 in the brain is derived from local conversion of T_4 into T_3 (8), and therefore, the relative activities of these two enzymes determine the levels of T_3 within the hypothalamus. To address this issue, Barrett *et al*. (6) assessed the status of these two key deiodinase enzymes in the Siberian hamster hypothalamus over the course of a natural seasonal cycle. Using *in situ* hybridization histology, their study showed that there was a seasonal cycle of Dio3 expression, with mRNA levels gradually rising over the course of exposure to short photoperiod, which peaked at approximately the time that complete regression of the testis had occurred. This suggests that the access of T_3 to key hypothalamic structures is gradually suppressed during exposure to short photoperiods, and that reduced T_3 initiates the switch to short-day physiology. Dio2 and Dio3 expression in the hypothalamus is strongly localized to the ependymal layer of the third ventricle, placing these enzymes in close proximity to structures implicated in seasonal neuroendocrine function, such as the dorsomedial posterior arcuate nucleus and paraventricular nucleus.

It is now becoming apparent that the regulation of thyroid activity in the brain has profound consequences for seasonal vertebrates. Interestingly, there exists species variation in whether it is the T_3 inhibitory pathway (Dio3) or stimulatory pathway (Dio2) that is under photoperiodic control. For example, the relative decrease in hypothalamic T_3 in short days is achieved in the Syrian hamster through a decrease in Dio2, whereas the Siberian hamster exhibits an increase in the expression of Dio3 in short photoperiod (6). Moreover, the period of reproductive quiescence in seasonal breeders appears to be associated with reduced T_3 availability. In contrast to the long-day breeding hamsters, the Saanen goat, a short-day breeder, exhibits increased Dio2 expression under short day photoperiod (5). Freeman *et al*. (9) also have re-
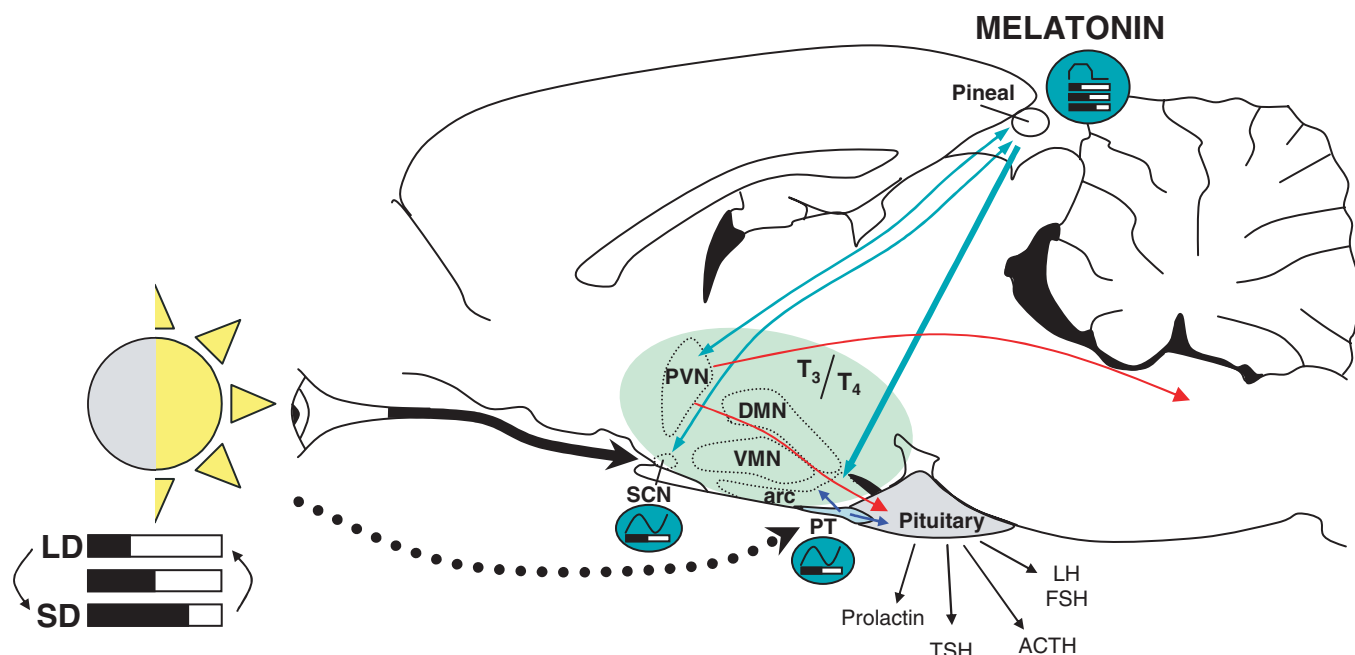
FIG. 1. In mammals, nightly melatonin secretion by the pineal is dictated via links with the master circadian clock, the SCN, which itself receives photic information from the retina. Melatonin signals impart circannual timing signals to the hypothalamus and pituitary, employing thyroid hormone modifying enzymes within the hypothalamus as a key intermediary. T_3 levels in the brain are dependent on the photoperiodic responsiveness and relative activities of T_3-producing (Dio2) and T_3-inactivating (Dio3) deiodinase enzymes (see text). Significantly, pinealectomy does not block responses to photoperiod in birds, raising the possibility that thyroid hormone signaling represents the ancestral mechanism employed by vertebrates to integrate time and neuroendocrine function. Two structures that appear to be central to this pathway are the ependymal lining of the third ventricle, and the PT. Tanycytes within the third ventricle ependyma are perfectly placed to relay information from the CSF and portal blood supply to the hypothalamic parenchyma (and possibly back again). Through the expressions of Dio2 and Dio3, tanycytes can influence T_3 levels throughout the hypothalamus (*green shading*). Notable targets of thyroid hormone within the hypothalamus include thyrotropin-releasing hormone neurons and GnRH neurons. The PT is known to be critical for seasonal prolactin release, and like the tanycytes, is well placed to serve as a relay point for circannual signals between the pituitary and key areas of the hypothalamus, possibly sending signals via the CSF and hypophyseal blood supply. Given that neither lesioning of the SCN nor pinealectomy block the photoperiodic changes in the gonads of some birds (22, 23), perhaps the PT might have once been directly responsive to photic cues. LD, Long day; SD, short day; PVN, paraventricular nucleus; DMN, dorsomedial nucleus; VMN, ventromedial nucleus; arc, arcuate nucleus.

cently demonstrated that daily peripheral injections of T_3 can induce gonadal growth in short-day housed Siberian hamsters. The role of T_3 in birds has been confirmed by studies in which high levels of T_3 were artificially maintained in the brain to circumvent the effects of photoperiod. Specifically, intracerebroventricular infusion of T_3 has been shown to stimulate testicular growth in short-day housed quails, whereas infusion of the Dio2 inhibitor, iopanoic acid attenuated testicular growth in long days (10). Taken together with the current study by Barrett *et al.* (6), we now have a model emerging in which seasonal changes in expression of deiodinase enzymes may provide a crucial link between photoperiod and downstream neuroendocrine pathways.

Localization of deiodinase expression to the ependymal layer of the third ventricle suggests that this structure is important in seasonal time keeping. Photo-responsive expression in the third ventricle ependyma has been demonstrated for a number of genes, including the melatonin-related receptor (GPR50) and retinoic acid-binding protein 1 (11). The ependyma is populated by specialized glial cells known as tanycytes, which are the primary source of Dio2 activity in the hypothalamus (12). These cells are thought to relay information between the cerebrospinal fluid (CSF), portal blood supply, and hypothalamic parenchyma, possibly

integrating hormonal, neuronal and even nutrient signals. Tanycyte processes exhibit significant morphological interactions with GnRH neurons in the hypothalamus (12). These contacts are responsive to photoperiod in the Siberian hamster (13, 14), and restructuring of tanycyte processes associated with GnRH neurons in short days has been suggested as a key feature regulating the attenuation of GnRH release and testicular regression in these animals (13). Impressively, exogenous T_3 administration in quail mediobasal hypothalamus can block photoperiodic-dependent changes in neuron/tanycyte interactions (13).

We are still far from knowing how melatonin regulates gradual programmed changes in deiodinase expression. Melatonin levels in the third ventricle CSF are higher than those in the circulation and reflect nocturnal duration (15). Yet there is no evidence that melatonin receptors are expressed in the ependymal cells (16). The actions of melatonin must, therefore, rely on an intermediate pathway. Perhaps the melatonin-related receptor (which does not bind melatonin) could be a candidate, as this orphan G protein receptor is expressed strongly in the ependymal layer, and also is strongly seasonal in expression (11). The densest expression of melatonin receptors in the region is located in the pars tuberalis (PT), a portion of the pituitary stalk that runs from

the hypothalamus down into the pituitary gland. The PT has immediate access to the hypophyseal blood supply and the CSF, and lies in relatively close proximity to the third ventricle. Melatonin signaling in the PT is sufficient to control seasonal changes in prolactin release (17, 18), and clock gene expression within the PT is influenced by melatonin (19). The PT is well placed to serve as a relay to the pituitary and key areas of the hypothalamus, perhaps via the CSF, and studies in sheep and other seasonal mammals suggest that the PT is central to the translation of melatonin signaling into seasonal physiology (20, 21).

Significantly, melatonin has little effect on seasonal physiology in birds, and in contrast to mammals, pinealectomy does not block responses to photoperiod. Therefore, the discovery that seasonal phenotypes in both birds and mammals are dependent on the regulation of deiodinase pathways raises the intriguing possibility that this is the ancestral mechanism employed by vertebrates to integrate time and neuroendocrine function. Thyroid hormones are implicated in the initial development and plasticity of the brain, so seem perfectly placed to coordinate the gradual, long-term changes in neural processing and neuroendocrine function that are a hallmark of seasonal physiology in adult mammals.

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Acknowledgments

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