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Review article

Melatonin and the seasonal control of reproduction

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Abstract — Many mammalian species from temperate latitudes exhibit seasonal variations in breeding activity which are controlled by the annual photoperiodic cycle. Photoperiodic information is conveyed through several neural relays from the retina to the pineal gland where the light signal is translated into a daily cycle of melatonin secretion: high at night, low in the day. The length of the nocturnal secretion of melatonin reflects the duration of the night and it regulates the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Changes in GnRH release induce corresponding changes in luteinising hormone secretion which are responsible for the alternating presence or absence of ovulation in the female, and varying sperm production in the male. It is not yet known where and how this pineal indoleamine acts to exert this effect. Although melatonin binding sites are preferentially localised in the pars tuberalis (PT) of the adenohypophysis, the hypothalamus contains the physiological target sites of melatonin for its action on reproduction. Melatonin does not seem to act directly on GnRH neurons; rather it appears to involve a complex neural circuit of interneurons that includes at least dopaminergic, serotonergic and excitatory aminoacidergic neurons. © Inra/Elsevier, Paris

melatonin / reproduction / photoperiod / prolactin / dopamine

Résumé — **La mélatonine et le contrôle saisonnier de la reproduction.** Un grand nombre de mammifères originaires des latitudes tempérées présentent des variations saisonnières d'activité sexuelle qui sont sous le contrôle du cycle photopériodique annuel. L'information photopériodique transite par plusieurs relais de la rétine à la glande pinéale où le signal lumineux est traduit en rythme nycthéméral de sécrétion de mélatonine : élevée le jour, faible la nuit. La durée de sécrétion nocturne de mélatonine est proportionnelle à la durée de la nuit et contrôle la sécrétion pulsatile de gonadotropin releasing hormone (GnRH). Les changements de libération de GnRH induisent des variations correspondantes de sécrétion d'hormone lutéinisante qui à leur tour sont responsables de l'alternance entre périodes ovulatoire et anovulatoire chez la femelle et des variations de production spermatique chez le mâle. Les sites et les mécanismes d'action de la mélatonine qui sont responsables de ces effets ne sont pas connus avec précision. Bien que les sites de liaison de la mélatonine soient localisés préférentiellement dans la pars tuberalis (PT) de l'hypophyse antérieure, l'hypothalamus contient les

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cibles physiologiques de la mélatonine pour son action sur la reproduction. La mélatonine ne semble pas agir directement sur les neurones à GnRH. Au contraire, son action implique un circuit complexe d'interneurones qui inclut au moins des neurones dopaminergiques, sérotoninergiques et à acides aminés excitateurs. © Inra/Elsevier, Paris

mélatonine / photopériode / reproduction / hypothalamus / dopamine

1. INTRODUCTION

In temperate zones, many mammalian species display a seasonal pattern of reproduction [7]. Depending on the time of year when they are sexually active, species are characterised as short-day breeders, for instance sheep, or long-day breeders, for instance hamsters or horses. The pineal hormone, melatonin, through its nocturnal mode of secretion, has been shown to be the primary transducer of photoperiodic information to the neuroendocrine reproductive axis [1, 2]. The importance of the pineal gland was demonstrated by numerous experiments showing that the effect of photoperiod on seasonal functions is profoundly altered in pinealectomised animals. In Syrian hamsters, pinealectomy prevents the seasonal reduction in gonadotropin secretion and the gonadal regression normally brought about by experimental or natural short photoperiod [55]. Injection of melatonin reverses the effect of pinealectomy on gonadotropin secretion and causes gonadal regression. This early work, mainly in the hamster, led to the concept of the antigonadal action of melatonin. However, more recent data, particularly those obtained in short-day breeders have led to a more generalised role of melatonin in the control of seasonal rhythms [26, 27, 29]. Specifically, pinealectomy suppresses responses to both short and long photoperiod, and melatonin, depending on its specific pattern, reinstates both these responses. Thus, the role of melatonin is to provide an endocrine code for daylength. This role was well illustrated by replacement studies in pinealectomised animals.

Melatonin delivered into the peripheral circulation to mimic short-day or long-day profiles can reproduce the stimulatory effects of short days or the inhibitory ones of long days on LH secretion in the ewe [29].

2. GnRH NEURONS AS THE FINAL NERVOUS PATHWAY OF MELATONIN ACTION

The duration of melatonin secretion is processed neurally to regulate the secretion of GnRH. The final step of the action of melatonin at the level of the central nervous system is a modulation of GnRH secretion, partly as a consequence of the modification of steroid feedback. Melatonin treatment causes an increase in the immunoreactivity of GnRH in cell bodies located in the hypothalamus and increased density of GnRH-containing elements in the median eminence, in addition to its suppressive effects on gonadal function in white-footed mice [17, 18]. In Djungurian hamsters, a rapid and transient increase in the distribution of detectable GnRH mRNA-containing cells is observed 2 days after transfer to stimulatory long days. This increase may be an early step in the stimulation of FSH secretion and gonadal growth which occur days later [3]. In sheep, it is possible to measure directly and reliably the release of GnRH in the hypophyseal portal system [9], and melatonin, given as a short-day profile, causes an increase in the frequency of pulsatile GnRH release ([69], *figure 1*). This increase in GnRH secretion is observed after a long lag time, i.e. 40–60 days, when LH

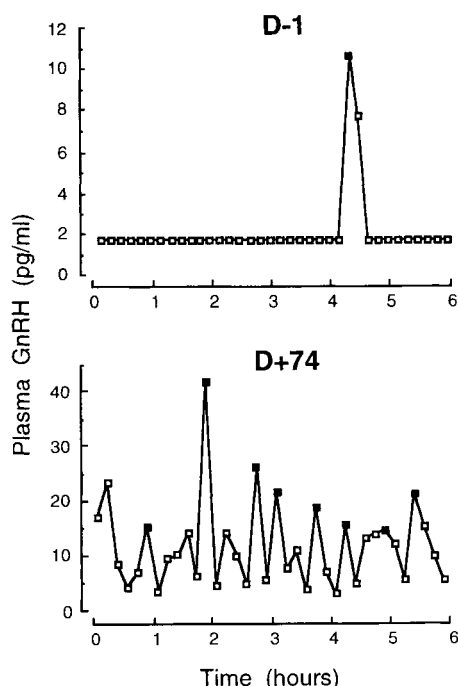


Figure 1. Effect of melatonin on GnRH pulsatile secretion. Examples of individual profiles of GnRH secretions before (D -1, top) or 74 days after (bottom) the insertion of a melatonin implant. Ewes were ovariectomised and treated with a subcutaneous estradiol implant and were exposed to long days (16L:8D). Blood samples were obtained every 10 min for 6 h on each occasion. The closed symbols depict detected pulses (adapted from [69]).

secretion is stimulated. Despite this effect of melatonin on the GnRH system, it is thought that melatonin does not act directly on the GnRH neurons; rather it acts indirectly through some interneuronal route that finally synapses on the GnRH neurons. The changes in GnRH secretion are the consequences of two complementary mechanisms controlled by melatonin [53]: a direct steroid-independent modulation of GnRH secretion and a change in the steroid negative feedback on GnRH secretion. For example, in ovariectomised (OVX) ewes, LH pulse frequency is lower in animals treated with a long-day profile of melatonin than with a

short-day one (one versus two pulses per hour) [51, 57]. This change in LH pulsatility between these two situations is increased dramatically when OVX ewes are treated with estradiol (E). Indeed, in OVX ewes treated with an E implant that elevates blood E levels to those of follicular phase intact ewes, one pulse is observed every 12–24 h in long days and every 30 min in short days [29].

3. SITES OF ACTION OF MELATONIN

The identification of the sites of action of melatonin is difficult since melatonin influences many physiological functions [1]. Melatonin could either act at a single site in the brain or pituitary which would then be implicated in the regulation of many seasonal functions, or melatonin could act at multiple sites, each involved in regulating one seasonal function. This difficulty is expanded by the localisation of high-affinity melatonin receptors in a wide variety of tissues in the body, although whether melatonin acting at these sites could affect seasonal functions remains debatable [5, 6, 52, 59].

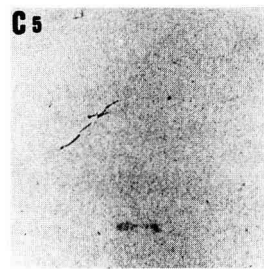
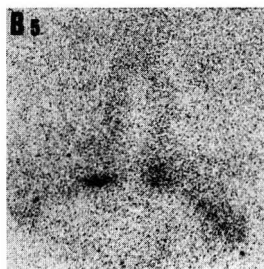
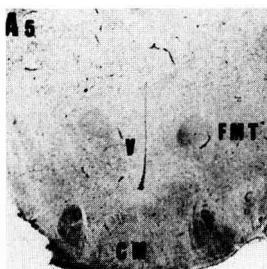
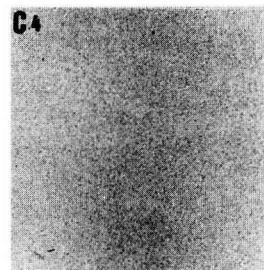
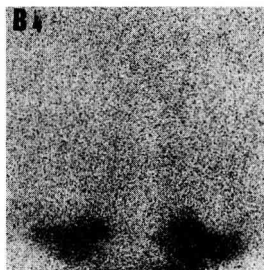
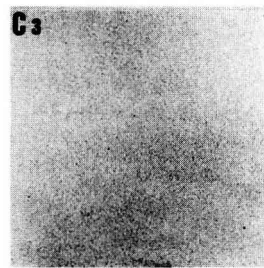
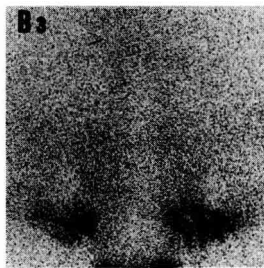
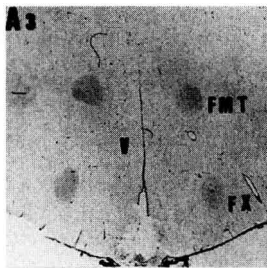
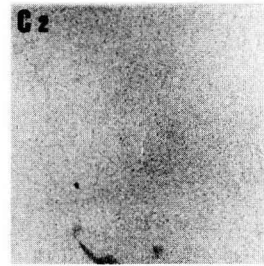
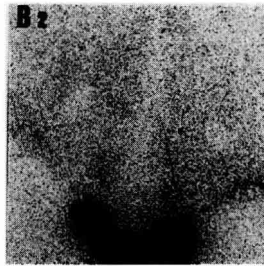
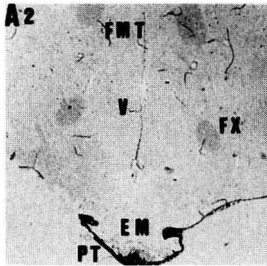
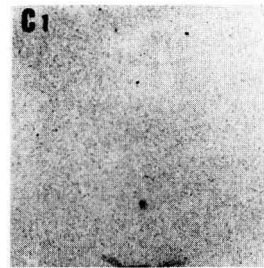
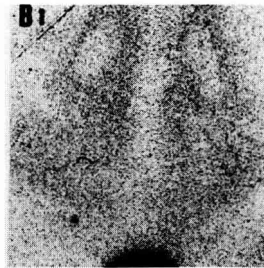
The first demonstration that melatonin acted in the brain was provided by studies in the white-footed mouse. Small beeswax pellets impregnated with melatonin and implanted in the anterior hypothalamus and medial preoptic area cause gonadal regression similar to that induced by short days. Pellets placed in other areas of the brain and subcutaneously have no effect on the gonads [19]. Interestingly, similar results were obtained with a rhythmic pattern of intracerebral melatonin delivery. Melatonin delivered for 10 h, but not 5 h, in the anterior hypothalamus or medial preoptic area causes gonadal regression of pinealectomised white-footed mice [13]. Similar results were obtained in Djungurian hamsters and gerbils [12, 22]. However, these studies could not define a precise target within the brain.

A major breakthrough was made with the development of the melatonin probe, ^{125}I -melatonin which allowed identification of putative target sites of melatonin within the hypothalamo-hypophyseal system whose importance was then tested by functional studies, mainly in hamsters and sheep. Although binding was found in several areas within the brain and the pituitary [6, 11, 68], it was the pars tuberalis that drew most attention since the density of binding was much higher than in any other hypothalamic or pituitary site and because it is a consistent feature among species investigated to date. Several studies have also found a seasonal variation in the melatonin binding site density and in the cytology of the pars tuberalis [15, 58]. Finally, the pars tuberalis is in a prime anatomical position for interacting with both the median eminence and the pars distalis of the pituitary and could, therefore, influence hypothalamo-hypophyseal function.

Surprisingly, studies in sheep and hamsters have led to the conclusion that the pars tuberalis does not mediate the action of melatonin on the neuroendocrine reproductive axis. Indeed, in the ewe, melatonin delivered directly to the PT does not appear to modify the secretion of LH: neither the placement of a melatonin microimplant directly against the anterior face of the PT [43] nor the discrete insertion of a microimplant into the PT [46] modified LH secretion. In contrast, microimplants placed in the MBH or third ventricle stimulated LH release [43, 46]. These studies provide definitive evidence that the hypothalamus, and not the PT, is the important melatonin

target for transducing the effects of this indoleamine on the reproductive neuroendocrine axis. Moreover, the placement of microimplants in the PT or pars distalis of Soay rams led to similar conclusions [41]. In that study, PT microimplants stimulated FSH secretion but the effect was much weaker than that obtained with MBH implants in a previous study, suggesting that the small effect observed with the PT implants resulted from a passive diffusion of melatonin to the MBH [39, 41]. In this species, melatonin binding was found in the premammillary hypothalamic area: it is located at the base of the brain and limited dorsally by the fornix; it extends 3 mm on either side of the third ventricle, is posterior to the infundibular recess, and is delimited caudally by the mammillary bodies ([10, 44], *figure 2*). A clear relationship was observed between the proximity of melatonin microimplants to the area of binding and the effectiveness of these microimplants to stimulate LH, strongly suggesting that melatonin targets are located in the premammillary hypothalamic area [44]. In hamsters, the hypothalamic localisation of the target sites of melatonin for acting on reproduction was also strongly suggested. Lesions of the dorsomedial hypothalamus melatonin binding sites block the gonadotropic response of male Syrian hamsters to short photoperiod or melatonin [48]. Furthermore, an overlap between melatonin binding and androgen receptor immunoreactivity is found in the dorsomedial nucleus and it was suggested that sensitivity to steroid feedback, a key mechanisms of the action of melatonin on gonadotropin secre-

Figure 2. Coronal sections of sheep hypothalamus illustrating distribution of melatonin binding sites. Top to bottom reflects rostral to caudal sequence (1–5). Left column (A): cresyl violet-stained sections; middle column (B): autoradiographic image of total iodomelatonin binding; right column (C): non-specific binding, estimated by incubating adjacent section with a 200-fold excess of cold melatonin. Note the specific binding of melatonin in the pars tuberalis (B1 and B2) and in the premammillary hypothalamic area (B3 and B4). Nomenclature: V: third ventricle; FMT: fasciculus mamillothalamicus; Fx: fornix; TO: tractus opticus; CM: corpus mamillare; EM: median eminence; PT: pars tuberalis (from [44]).



tion, might be influenced in this area in Syrian hamsters [47]. Although it remains to be established whether the areas identified in hamsters and sheep are functionally related, these data suggest a discrete target for melatonin in the hypothalamus.

The pars tuberalis mediates, at least in part, the action of melatonin on prolactin secretion. Indeed, in the ewe, melatonin implants inserted within the pars tuberalis, have an inhibitory effect on prolactin release [46]. Also, in rams with a surgical hypothalamo-pituitary disconnection in which the central control of prolactin secretion is abolished, the ability of melatonin to inhibit the secretion of prolactin is maintained [40]. Interestingly, in hamsters, lesion of the melatonin binding sites in the medial basal hypothalamus suppresses the effect of melatonin on the reproductive axis, but does not do so on prolactin secretion [48].

The dual site of action of melatonin for controlling gonadotropin and prolactin secretion is interesting in relation to the photoperiodic regulation of these two functions. Indeed, the action of photoperiod and melatonin on reproductive activity is characterised by long latency and the importance of photoperiodic history which is consistent with an action at the level of the brain for the storage of information. In contrast, photoperiodic history is not critical to the regulation of prolactin secretion [21] and the action of melatonin is relatively rapid (only a few days [73]) which is consistent with a more 'classical' endocrine regulation at the level of the pituitary. This regulation could involve tuberalin, a putative protein produced by the target cells of melatonin, that would influence gene expression and secretion in lactotrophs [25].

In mammals, two subtypes of high affinity G-protein-coupled melatonin receptors have been cloned (Mel-1A and Mel-1B) [56]. The high level of expression of these receptors in the pars tuberalis suggests that they mediate the action of melatonin on prolactin secretion. In contrast, the photore-

sponsiveness of hamsters in which the Mel-1B receptor gene cannot encode a functional receptor [74] and the failure to obtain a strong expression of these receptors in the hypothalamus outside of the suprachiasmatic nucleus raise the question of the type of melatonin receptors involved in the control of reproductive activity.

4. INTERNEURONS BETWEEN MELATONIN TARGET NEURONS AND GnRH NEURONS

Several pieces of evidence suggest that melatonin does not act directly on GnRH neurons. First, the distribution of most GnRH neurons does not match that of the putative sites of action of melatonin. In sheep, most GnRH neuronal perikarya are located in the preoptic area (60 %) with a few located in the MBH (15 %); some of these project to the median eminence and abut portal vessels [8, 38]; no GnRH neurons were identified in the pre mammillary area. Second, a large number of neurotransmitters are involved in the regulation of the GnRH neurons and constitute potential candidates to mediate the effects of melatonin on GnRH release. Indeed, experimental evidence implicating several neuronal systems has been obtained.

4.1. Dopamine

Many studies have suggested that dopamine is involved in transducing the negative feedback of E on GnRH secretion. For instance, systemic injection of a dopamine antagonist (pimozide) during anoestrus (strong negative feedback of E) induced a temporary increase in LH secretion [49]. Similarly, pimozide caused a temporary increase in LH secretion in long-day exposed (photo-inhibited) OVX + E ewes [33]. The A15 hypothalamic cell group appears to be a key dopaminergic structure involved in mediating the inhibitory effects of E since a neurotoxic lesion of this struc-

ture during anoestrus caused an increase in LH secretion [64]. Furthermore, it has been shown that E increases tyrosine hydroxylase (TH, rate limiting step enzyme of catecholamine synthesis) activity in long-day treated ewes [16] and induces *c-fos* gene expression in TH-immunoreactive cells of this structure in a season-dependent manner [36]. A similar role has been proposed for the A14 dopaminergic cell group [24]. Since a major effect of melatonin is a modulation of E negative feedback on GnRH secretion, this implication of dopamine makes these neurons likely candidates to act as relays between melatonin target sites and GnRH neurons [63].

In order to test this hypothesis, we first determined whether photoperiod and melatonin could modulate the activity of some dopaminergic neurons in the hypothalamus. Exposure to stimulatory short days resulted in decreased dopaminergic activity in the median eminence as assessed both by a reduction in dopamine content and in TH activity [62, 72]. No effect of short-day exposure on noradrenaline content in this structure or on TH activity in the other hypothalamic areas investigated was found [72]. The stimulation of LH secretion by a melatonin implant caused a parallel reduction in TH activity which suggests strongly that the effect of photoperiod on TH activity is mediated by melatonin [73]. The inhibition of median eminence TH activity by short days or by treatment with a melatonin implant is expressed at a time when the inhibition of prolactin secretion is already maximal, suggesting that these photoperiod-induced changes in TH activity are independent of the regulation of prolactin secretion [73]. Rather, they appear to be related to the photoperiodic regulation of LH secretion. Indeed, the pharmacological blockade of TH locally in the median eminence of ewes treated with 35 long days led to an increase in LH secretion [71]. This finding is consistent with the effect of pimozide implants in the median eminence of anoestrous ewes [23]. Interestingly, the pharmacological

blockade of TH activity no longer causes a stimulation of LH secretion when it is applied after a longer exposure to long days (71 days [4]). These data suggest, therefore, that an increase in TH activity in the median eminence is an important component of the inhibitory effect of long-day melatonin profile on GnRH output, at least for the initial phase of the inhibition. However, such modulation of TH activity appears to be E-independent because the photoperiod-induced changes in TH activity are similar in OVX and OVX + E ewes [72]. Thus, in contrast to the A15 and possibly A14 dopaminergic nuclei, which are involved in the modulation of the E negative feedback, the dopaminergic neurons of the median eminence appear to be involved upstream relative to the integration of the E signal. The median eminence in sheep is a structure rich in dopaminergic terminals but contains no TH-immunoreactive perikarya [65]. The localisation of the cell bodies projecting their axons to the median eminence, and more generally the anatomical and functional relationship between the A14 and A15 nuclei and the median eminence, have yet to be determined.

In male Syrian hamsters, exposure to inhibitory short days, presumably through melatonin secretion, reduces dopamine and noradrenaline turnover in the median eminence [60, 61]. This change is associated with a decrease in L-aromatic amino acid decarboxylase-positive cells in the arcuate nucleus without changes in TH-immunoreactive cell number or TH activity [31, 32]. Suppression of LH and FSH release in this species is presumably related to reduced noradrenaline activity, whereas reduced dopamine turnover may represent a consequence of suppression of prolactin levels by short days [60, 61].

4.2. Serotonin

Evidence for the implication of serotonin was also obtained in sheep. Serotonin inhibits LH pulsatile secretion during seasonal

anoestrus, and not during the breeding season [33, 50]. This inhibitory effect of serotonin is mediated by 5-HT_{2A} receptors [34] and interestingly a photoperiod-induced change in the density of 5-HT_{2A} receptors in the ventrolateral posterior hypothalamus was described [35, 54].

4.3. Excitatory amino acids

The implication of excitatory amino acids in the photoperiodic regulation of LH secretion was suggested by experiments involving administration of N-methyl-D,L-aspartic acid (NMDA), an agonist of neuroexcitatory amino acids. Such an administration acutely stimulates GnRH and LH secretion in seasonal species such as sheep and hamsters. However, this stimulatory effect is larger during periods of photoinhibited LH secretion than during photostimulated periods [28, 42, 70]. Also, in male hamsters, the inhibitory effect of short days on testicular activity can be blocked by chronic treatment with NMDA [66]. Although glutamate is involved in the photic regulation of circadian rhythm at the level of the retinohypothalamic tract, this action of NMDA cannot be explained by an effect on the melatonin secretory rhythm. Indeed, the differential effect of NMDA in photoinhibited and photostimulated ewes is maintained if stimulation is induced by melatonin administration instead of short-day exposure [70]. Furthermore, in Djungarian hamsters, the chronic effect of NMDA is observed on the photoperiod-induced changes in reproduction, but not on other functions (pelage colour, body weight [14]). These experiments suggest that a change in excitatory amino acid input may be part of the mechanism mediating the action of melatonin on GnRH secretion. Consistent with this hypothesis, photoperiod modifies the density of NMDA receptors in the preoptic area of hamsters [67].

Besides the neurotransmitters or receptors mentioned above, future research will

probably reveal the involvement of other neuronal systems in mediating the action of melatonin on GnRH neurons. Regardless of the nature of these neurons, the action of melatonin on GnRH neurons most likely involves a complex network of interacting neurons.

Part of the mode of action of photoperiod could be to cause morphological changes in this neuronal network. Evidence for such an effect was obtained at the level of the GnRH neurons of the ewe. At the light microscopy level, immunostained GnRH neurons in the preoptic area have longer and more numerous dendrites during anoestrus than during the breeding season [38]. However, these changes could reflect alterations in the content or localisation of immunodetectable GnRH, rather than actual morphological changes. At the electron microscopy level, GnRH neurons in the preoptic area receive more than twice the mean number of synaptic inputs per unit of plasma membrane during the breeding season as during anoestrus [37, 75]. Interestingly, these changes are not dependent on changes in ovarian steroid levels which differs markedly with the examples of seasonal plasticity described in the literature due to changing levels of steroids. The changes observed in the preoptic area of the ewe could, therefore, reflect the action of photoperiod and melatonin, or the expression of the circannual rhythm of reproduction, a key element of the photoperiod responsive mechanisms in this species [45].

In relation to possible phenomena of neuronal plasticity, it is worth noting that thyroid hormones have been strongly implicated in allowing seasonal changes in reproductive activity [30]. Reproductive transition from the breeding season to anoestrus is dependent upon the presence of thyroid hormones. Thyroidectomised ewes do not exhibit a seasonal decline in episodic secretion of GnRH in hypophyseal portal blood and thus fail to enter anoestrus. Thyroxine replacement reverses the effect of

thyroidectomy and the seasonal changes in thyroid hormone concentrations are not necessary for this reversal [30]. Since thyroid hormones are essential for the normal morphological maturation of the central nervous system [20], the permissive role of thyroid hormones for seasonal changes to occur may be to cause the prerequired morphological rearrangements for the changes in GnRH secretion.

5. CONCLUSION

The regulation of GnRH secretion by melatonin is central to the photoperiodic regulation of reproductive activity. The target sites of melatonin appear to be located in the hypothalamus but their precise localisation (at a cellular level) and their neurotransmitter phenotype have yet to be determined. Melatonin does not act directly on GnRH neurons; rather its action is probably mediated by a complex network of interneurons. The identification of the elements of this network and their inter-relationship will enable us to understand the long-term mechanisms that melatonin uses to control GnRH secretion.

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