REPRODUCTIVE BIOLOGY

Melatonin - a modulator of the GnRH/LH axis in sheep

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SUMMARY

The nocturnal secretion of pineal melatonin provides information to vertebrates on changes in day length under the circumstances in which they live. In sheep, which are seasonal breeders, the secretion of melatonin is also a signal to the neural structures controlling the secretion of gonadotropins from the pituitary gland to drive their activity in accordance with the season of the year. The sites and mechanisms of melatonin action on GnRH/LH secretion have been the subject of intensive studies in the last decade. This article briefly reviews the most important discoveries and methods used in this research, which has led to a better understanding of the role of melatonin in the modulation of hypothalamo-pituitary-gonadotropic axis activity in sheep. The identification of melatonin receptors within the central nervous system and in the pars tuberalis of the pituitary gland, as well as the use of specific techniques of micro-implantation and micro-infusion were crucial in this aspect. *Reproductive Biology 2002 2 (3): 267-275.*

Key words: melatonin, GnRH, LH, sheep, photoperiod

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INTRODUCTION

Sheep are domesticated animals, which in temperate latitudes still remain seasonal breeders. The photoperiod drives their reproductive cycle, which comprises a season of high sexual activity during short days and an anestrous season that occurs during long days. Information on changes in the photoperiod is provided to the organism through nightly secretion of melatonin from the pineal gland. Thus, changes in the duration of melatonin secretion constitute a signal to the neural structures controlling the secretion of gonadotropins from the pituitary gland that a long duration is stimulatory and a short duration is inhibitory. In numerous studies in which anestrous animals were treated with exogenous melatonin, it was demonstrated that a sustained high melatonin level in the organism led to the activation of the hypothalamo-pituitary GnRH/LH axis [1, 3, 8]. Activation of this gonadotropic system in anestrous ewes leading to the onset of estrus, requires, however, several weeks of exposure to melatonin, during which a change in the feedback action of estradiol on GnRH/LH plays a pivotal role [10]. It is not known exactly what the role of melatonin is in this transformation. Is melatonin only a signal that synchronizes this process in due time? Does melatonin modify the signals originated from other neural centers, e.g. dopaminergic or opioidergic, whose activity is related to gonadal steroids? Finally, does the pineal hormone directly modulate the secretory activity of the GnRH/LH axis?

Melatonin and the central nervous system

Intensive studies, especially at the brain level, have been conducted in the last decade to elucidate the ways of melatonin action on GnRH/LH secretion and to identify melatonin binding sites. The results obtained after placing micro-implants containing melatonin in different areas of the sheep brain focused attention on the mediobasal hypothalamus (MBH). Melatonin delivered into this structure induced gonadotropin secretion in most ewes [15] and rams [12], similarly as in case of subcutaneous implants [28]. For a long time, however, melatonin receptors were either not found in the sheep hypothalamus or were detected in small numbers in places that are not involved in reproduction. In the preoptic area, which is rich in GnRH--synthesizing cells, melatonin failed to stimulate the GnRH/LH axis [11, 12].

Finally, the use of autoradiography and micro-implantation made it possible to demonstrate that a discrete area of higher binding of melatonin is located in the premammillary hypothalamic area (PMH, fig. 1) and that melatonin is able to stimulate LH secretion if it is delivered into this site [18]. Importantly, LH secretion was stimulated in all animals with micro-implants placed correctly with respect to the higher binding area, suggesting that the PMH is an important target for melatonin in regulating reproductive activity in ewes. The easiness of diffusion of melatonin from the pineal recess of the third ventricle to its ventral part [27] indicates, in turn, that the pineal hormone may be caught by the PMH cells directly from the cerebrospinal fluid. The understanding of the mechanism of melatonin action on GnRH/LH secretion will require, however, identification of the melatonin binding cells within the PMH as well as the description of the pathways by which they may connect to GnRH neurons.

Melatonin and the pars tuberalis of the pituitary gland

Simultaneous studies on melatonin receptors in the pituitary gland showed that high-affinity melatonin-binding sites are present in the pars tuberalis (PT, fig. 1) The PT is a morphologically distinct and highly vascularised region of the pituitary, which connects the median eminence of the hypothalamus with the pars distalis (PD) of the gland [22]. In sheep, this adenohypophysial region has the greatest density of melatonin receptors, but does not seem to be a crucial target for the reproductive action of melatonin. Specifically, micro-implants of melatonin, positioned directly in the PT or apposed against its anteroventral face, did not modify the secretion of LH in comparison with the effectiveness of melatonin micro-implants placed in the third ventricle immediately above the MBH [16, 17]**.** On the other hand there is a strong evidence that the PT is involved in the seasonal control of another pituitary hormone – prolactin (PRL). This observation originated from experiments performed on hypothalamo-pituitary-disconnected (HPD) rams, in which the neuroendocrine communication between the hypothalamus and the pituitary was surgically eliminated [13]. The HPD rams exhibited well-defined seasonal cycles in prolactin secretion with high PRL concentration under long day conditions and low PRL concentration under short day conditions. Moreover, melatonin implants in HPD rams kept under long-day conditions caused a decrease in prolactin concentration similar to that in intact animals [13]. Due to the lack of melatonin receptors on lactotrophs, it is reasonable to suggest that the PT may mediate the observed effect of melatonin. One of the favored hypothesis states that the PT secretes a prolactin-releasing factor under long day conditions, which is inhibited by melatonin under short day conditions [21].

PT tissue contains several cell types including "PT-specific cells", classical granular secretory cells and folliculo-stellate cells [26]. The structure of "PT-specific cells", the major cell type in PT tissue, suggests a putative secretory function (fig. 2). In the Syrian hamster, glycoprotein hormone α-subunit and thyrotropin-stimulating hormone β-subunit are present in PT-specific cells and their expression undergoes photoperiod-dependent changes [6]. In contrast, the expression of β subunits of glycoprotein hormones in ovine PT-specific cells has not yet been proven. There is a report, however, concerning a factor called tuberalin, which is secreted by the ovine PT, that affects PD lactotrophs to increase *c-fos* gene expression and to stimulate prolactin release [23]. The secretion of tuberalin is enhanced by forskolin (adenylate cyclase activator) and the forskolin-stimulated tuberalin secretion is inhibited by melatonin [23]. This suggests that the endocrine effect of tuberalin is of importance in the pituitary mechanism of melatonin action, especially in regards to the control of the seasonal cycle of prolactin.

Gonadotrophs are the main defined endocrine cell type amongst granular secretory cells present in ovine PT tissue (fig. 2). It was shown that ovine PT secretes LH and that this secretion is enhanced in response to GnRH [25]. The response of the PT to GnRH was similar to that shown by the PD. Importantly, melatonin attenuated the GnRH-induced increase in LH secretion from the ovine PT, but not from the PD [25]. The population of gonadotrophs in the PT is probably too small for their LH to have a noticeable effect on circulating LH concentration. However, in some physiological stages, "melatonin-sensitive" LH, secreted by the PT, may act on the brain to influence the reproductive neuroendocrine axis via a short-loop feedback system. Although long-acting melatonin micro-implants placed in the PT did not affect the seasonal LH cycle [16, 17], it is reasonable to suggest that the pineal hormone may affect the circadian rhythm of this gonadotropin.

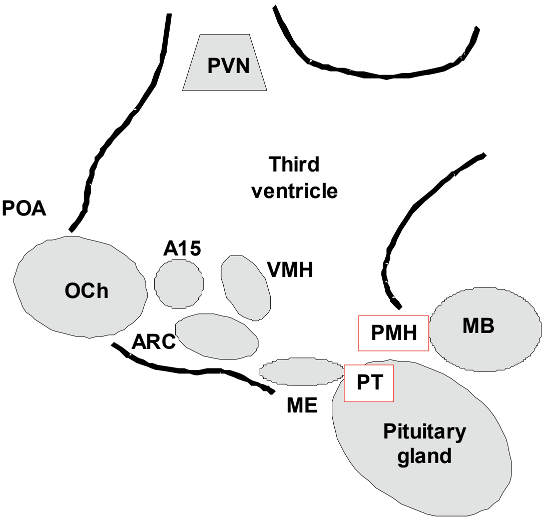

Fig. 1. Schematic drawing of the ovine hypothalamus showing the distribution of melatonin binding areas: PMH, premammillary hypothalamus and PT, pars tuberalis (red frame). Other visible brain structures: POA, preoptic area; OCh, optic chiasma; PVN, paraventricular nucleus; A15, dopaminergic A15 nucleus; VMH, ventromedial nucleus; ARC, arcuate nucleus; ME, median eminence; MB, mammilary bodies.

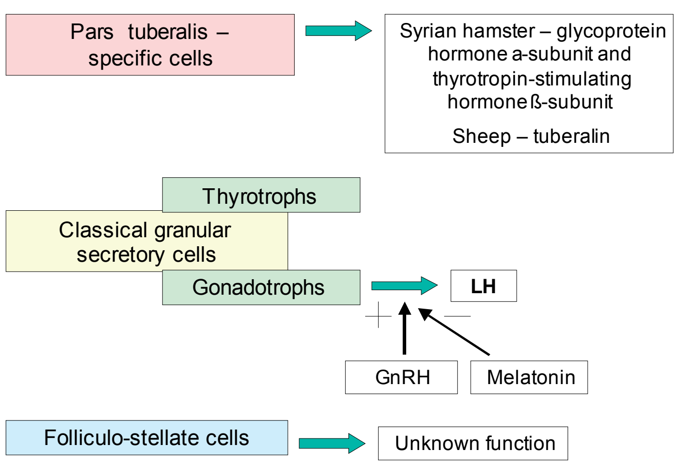

Fig. 2. The identified structures and products of the pars tuberalis cells. Gonadotrophs of the ovine pars tuberalis secretes LH in response to GnRH (*in vitro*). Melatonin attenuates the GnRH-induced increase in LH (see: Skinner and Robinson [25]).

Short-term effect of melatonin on the GnRH/LH axis

Some studies on sheep have reported changes in LH secretion related to light/dark phases. During a twenty-four hour cycle, mainly sexually active ewes [4] and rams [14] were reported secreting more LH at night. In our study, an increase in LH release was observed in luteal-phase ewes during the first hours of darkness. A similar, short-term enhancement in LH concentration was found in these ewes during the first hour of an afternoon intracerebroventricular (icv.) infusion of melatonin [20]. Taking into consideration the presumed effect of melatonin on LH secretion from the PT of the pituitary gland it could be expected that this secretion was suppressed by the infusion of melatonin. This might be in turn a stimulus for transient hypothalamic GnRH release and consequently secretion of LH from the gonadotrophs of the PD. Such a mechanism would function during the breeding season to intensify LH secretion and for this reason deserves further investigation. In contrast, short-term melatonin administration into the ewes' central nervous system did not stimulate GnRH and LH release during seasonal anestrus [24]. This is in agreement with the view that the GnRH/LH axis in anestrous ewes is strongly inhibited and insensitive to the brief administration of the pineal hormone. Interestingly, in both breeding and anestrous seasons, ovariectomy-induced LH secretion was suppressed by melatonin infused icv. [20, 24]. Melatonin regained its short-term stimulatory effect after ewes, ovariectomized (OVX) during the breeding season, were injected intramuscularly for several days with estradiol [20]. It is generally accepted that the increased activity of the GnRH/LH axis during the breeding season is derived endogenously [9] and is synchronized by the melatonin signal with the seasons of the year [29]. However, it appears that a central interaction between melatonin and estradiol is needed to sustain a high level of LH secretion during the reproductive period.

During seasonal anestrus, the inhibition of pulsatile LH secretion by estradiol is mediated by the dopaminergic network [2, 5]. Inspired by results of our earlier study in which melatonin had suppressed pulsatile LH release during steroid deprivation [24], subsequently we have tested a hypothesis that the pineal hormone may exert a similar effect after direct elimination of inhibitory dopaminergic input (T. Misztal, unpublished data). In this study, a several-hour icv. infusion of melatonin in anestrous ewes was preceded by the subcutaneous injection of sulpiride, a dopamine (DA) antagonist. We found that melatonin indeed suppressed LH pulse frequency followed by pharmacological blockade of DA receptors with sulpiride (T. Misztal, unpublished data). The exact mechanism of this intriguing action of melatonin is unclear but it may involve the hypothalamus and/or the pituitary gland. Molecular studies on sheep pars tuberalis, a major neuroendocrine target site for melatonin, have shown, however, that melatonin alone has no direct effect on a number of intracellular signal transduction processes. Melatonin instead seems to either prevent or reverse the effects of different stimuli on second-messenger systems [7, 19]. Thus, in our case, it is possible that melatonin affects the intracellular DA signal transducing pathway which is disturbed following sulpride treatment. Moreover, it is well known that the seasonal changes in dopaminergic network activities are elements of the feedback action of estradiol [2, 5]. Thus, the DA-involving mechanism may also mediate the long-term effect of melatonin on reproductive activity in ewes.

Despite the efforts of many research groups it is still not fully understood how melatonin affects the GnRH/LH axis. However, our present understanding shows the complexity of the photoperiod related reproductive processes in sheep and challenges future investigations.

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