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Reproductive Physiology of Pineal Hormone Melatonin

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Melatonin is a multifunctional hormone secreted from the pineal gland in the middle of cerebrum and cerebellum. Its synthesis and release reflect photoperiod. Photoperiod is a yearly predictable ambient factor that most animals utilize as an environmental cue for maximum survival. Hamsters maintain reproductive activity in summer during which day length exceeds night time. Upon the advent of autumnal equinox they undergo gonadal regression. The photoperiodic effects are prevented by removal of the pineal gland and restored by the timed replacement of melatonin. The results suggest that melatonin constitutes part of control mechanism whereby environmental information is transduced to neuroendocrine signal responsible for the functional integrity of the reproductive system. From the studies for the action site of melatonin following the treatment of photoperiod or melatonin in the lesion of a specific portion of hypothalamus, suprachiasmatic nuclei and pars tuberalis are shown to be a consensus site for melatonin. The action of melatonin in the regulation of reproduction is largely unknown. It is mainly due to the lack of acute effect of melatonin on gonadotropin secretion. However, reduction of the gonadotropin release and augmentation of the hypothalamic gonadotropin-releasing hormone (GnRH) content by long-term treatment of melatonin indicate that constant presence of melatonin may participate in the regulation of sexual activity via the GnRH neuronal system. The action mechanism by which melatonin exerts its effect on GnRH neuron needs to be elucidated. The inability of opioid analogues to affect the reproductive hormones in sexually regressed animals by inhibitory photoperiod and melatonin suggests that the opioidergic neuron may be a prime intervening mediator. Recent cloning of melatonin receptor will contribute to investigate its anatomical identification and the action mechanism of melatonin on target tissues at the molecular level.

KEY WORDS: Melatonin, Opioid, Pineal, Reproduction, Seasonal Breeding, Gonadotropin-releasing Hormone

The pineal gland had long been considered as a vestigial organ until melatonin was isolated and characterized from the beef pineal gland (Lerner *et al.*, 1958). Although the earlier report that extracts of the pineal gland were able to cause blanching of tadpole skin (McCord and Allen, 1917) marked the inception of melatonin research, the activity of melatonin as a pigment-concentrating hormone remains an important physiological activity, at least in lower vertebrates. The discovery of the chemical structure of melatonin in mammals led to the realization of many other of its biological activities.

These include its action as a mediator of

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photoperiod in regulating seasonal reproduction (Stetson and Watson-Whitmyre, 1986), a biological clock to time circadian rhythmicity (Stetson and Watson-Whitmyre, 1976) and a neuromodulator in the retina (Dubocovich, 1983). In addition, there are a great deal of increasing suggestions that the pineal gland through the melatonin is potentially involved in the anti-aging and life-prolonging effects relating to the antioxidative mechanism (Tan *et al.*, 1993), appears to exert an inhibitory effect on the growth of tumor and cancer cells (Dogliotti *et al.*, 1990), participates in the control of cardiovascular function as an endogenous hypotensive/bradycardiac factor in the central nervous system (Chuang *et al.*, 1993), and plays a supportive role in the immune system (Maestroni *et al.*, 1987). Indeed, melatonin has multifunctional roles at various levels of molecule, cell, tissue, organ, system, and whole body.

Among the action of the pineal and melatonin, much concerns have been taken on the regulation of reproductive activity. In order to assure that each generation of a species survives to the next, most animals in temperate zone have developed a "reproductive strategy," which is seasonality because they are subjected to yearly fluctuation of ambient photoperiod, temperature, precipitation, and food availability. Photoperiod is the most important environmental cue used to time reproductive activity, although other species may also use additional factors. A major reason that reproduction is dependent on the changing day length is that this factor is predictable from one year to the next. Although other environmental factors also change throughout the course of each year, their stability is not as precise as is photoperiod. An annual cycle of reproductive activity endows organisms with the advantage of confining birth to a time of year when chances for survival are optimized. The effect of photoperiod on the reproductive activity is accurately reflected by the melatonin rhythmicity (Stetson and Watson-Whitmyre, 1986). The present review summarizes the synthesis and neural control of pineal hormone melatonin and the influence of pineal and melatonin on reproduction of seasonal breeding animals focusing on the male hamsters.

Melatonin Synthesis

Melatonin (N-acetyl-5-methoxytryptamine) is a product of tryptophan metabolism by the pineal gland (Fig. 1). The pineal gland (epiphysis cerebri) is believed to uptake tryptophan into the pinealocyte by a neural amino acid transport mechanism (Sugden, 1989). Tryptophan is converted by tryptophan hydroxylase to 5-hydroxytryptophan, which is decarboxylated by aromatic amino acid decarboxylase to form 5-hydroxytryptamine (serotonin). Serotonin is transformed to N-acetylserotonin by the action of N-acetyltransferase. Hydroxyindole-O-methyl transferase produces melatonin from the N-acetylserotonin. Melatonin is then metabolized in the liver to 6-hydroxymelatonin by melatonin hydroxylase and converted into a sulfate or to a glucuronide for urinary excretion (Sugden, 1989).

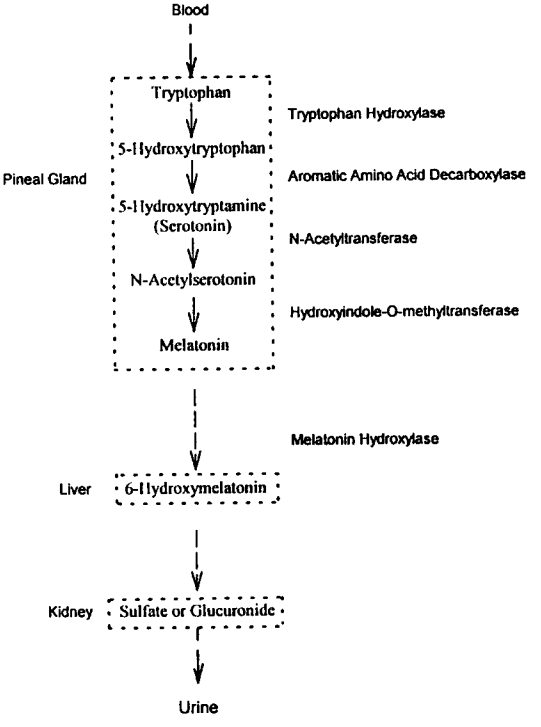

Fig. 1. Biosynthesis of melatonin and its metabolism. Organs are marked with dotted boxes with their names at left side. The dotted arrows represent transportation through blood circulation or urine. Enzymes at the right side of the solid arrows in the pineal gland and an enzyme in the liver convert the above to the below.

Melatonin synthesized in the pineal gland is believed to be immediately released into the systemic circulatory system because there is a parallel correlation of melatonin level in serum and pineal gland (Maywood *et al.*, 1993). In addition, there is no portal system between the pineal and other brain areas in mammals examined so far (Chunhabundit and Somana, 1991).

Photoneural regulation of melatonin synthesis

In all mammals, the duration of elevated pineal and serum melatonin is proportional to the length of dark period (Stetson and Watson-Whitmyre, 1986). When the animals are moved from long photoperiod (LP, short night) to short photoperiod (SP, long night), the pineal melatonin content expands gradually (Hastings *et al.*, 1987). Even in constant darkness, the animals show a similar pattern of melatonin production observed in the animals in light-dark cycle (Tamarkin *et al.*, 1980). The exposure of animals to light at night when melatonin levels are high abruptly curtails pineal melatonin production and causes a rapid decline in tissue and blood levels of the hormone (Rollag *et al.*, 1980b). These observations indicate that melatonin synthesis within the pineal is controlled by the light.

The photic signal reaches the pineal by a multisynaptic pathway from the ocular photoreceptors (Fig. 2). The retinohypothalamic tract transmits the light message from the eyes to the suprachiasmatic nuclei (SCN), which send neuronal projections to paraventricular nuclei (PVN) of the hypothalamus. From the PVN the signal travels to the spinal cord via brain stem and exits via the thoracic preganglionic sympathetics. These synapse on postganglionic fibers in the superior cervical ganglia (SCG). The pineal gland is innervated by these postganglionic sympathetic neurons (nervi conarii) projecting from the SCG. All of the neurotransmitters involved in this long multisynaptic pathway from retina to pineal have not yet been identified, though some are known. A factor affecting the synthesis of melatonin is norepinephrine released from the axonal terminal of the postganglionic sympathetic neurons present within the pineal gland (Møller, 1992). Disruption

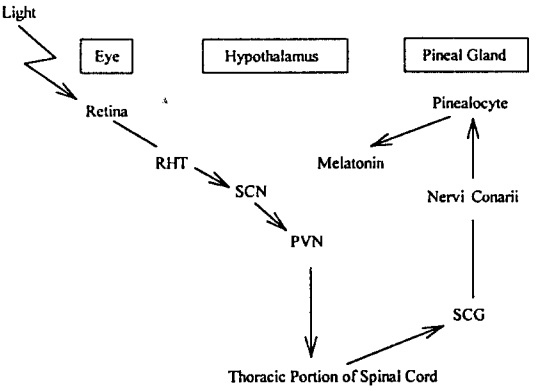

Fig. 2. Photoneural pathway that leads to melatonin production in the pineal gland. RHT, retinohypothalamic tract; SCN, suprachiasmatic nuclei; PVN, paraventricular nuclei; SCG, superior cervical ganglion.

of the neuronal pathway at any point between the retina and the pineal alters the pattern of melatonin production. Pinealocytes have been shown to have β-adrenergic receptors and α-adrenergic receptors that bind catecholamines (Romero and Axelrod, 1974; Sugden *et al.*, 1985). Thus, the pineal gland is considered to be a neuroendocrine transducer, as neural input to this organ is converted into an endocrine output.

Action of melatonin on reproduction

Representative animals demonstrating seasonal breeding pattern are hamsters, which are intensively studied. The Syrian hamster utilizes the photoperiod as an environmental cue, leading to the annual reproductive cycle. The annual cycle of reproductive capability is conveniently categorized into four phases; the photosensitive phase, regressing phase, regressed phase, and photorefractory phase (Fig. 3). Under natural conditions in the temperate latitudes, adult Syrian hamsters are reproductively active spring and summer until the autumnal equinox in mid-late September. In the fall, hamsters experience gonadal regression as the photoperiod shortens (Reiter, 1980a; Brainard *et al.*, 1984). In addition, since the hamsters are hibernators during the winter season, spending great amounts of time in their subterranean habitat, they are exposed to gradually fewer and fewer hours of daylight. This results in gonadal atrophy. In late winter, the

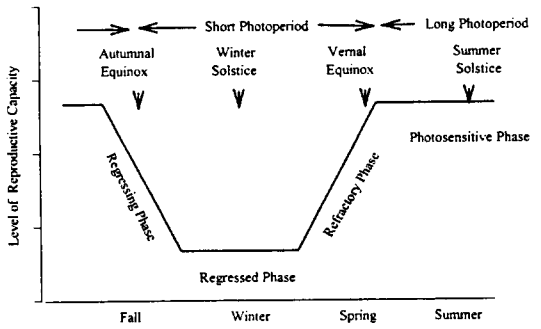

Fig. 3. A schematic representation of the annual reproductive cycle of male Syrian hamsters (*Mesocricetus auratus*) from natural daylength and laboratory experiments.

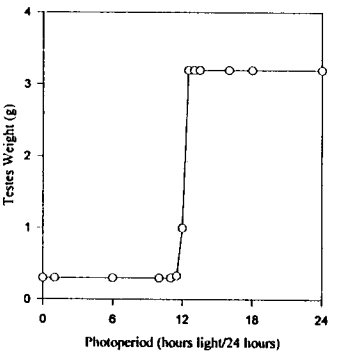

Fig. 4. A simplified diagram of testicular response to various photoperiods in laboratory with constant temperature (Elliott, 1976).

gonad begins to recrudesce, but not as a consequence of increasing daylength (Reiter, 1980b). Exposure of animals to constant darkness at this time of the year does not suppress or delay gonadal recrudescence (Reiter, 1975) and the neuroendocrine-gonadal axis is referred to as refractory to photoperiod. Therefore, hamsters are capable of reproducing immediately when they emerge from their hibernaculum. This annual cycle of reproduction increases the likelihood of survival.

The seasonal change in the reproductive status of the hamster can be reproduced in the laboratory during any season of the year with carefully controlled lighting adjustment. Under experimental conditions, adult male hamsters maintain reproductive activity when exposed to LP ($\geq$12.5 hours of light per day) including constant light, while SP (<12.5 hours of light per day) including constant darkness causes the testes to regress (Fig. 4, Elliott, 1976). Light deprivation by enucleation leads to testicular regression (Reiter and Hester, 1966). Exposure of an animal with regressed testes to photoperiods greater than or equal to 12.5 hours of light causes testicular regrowth (Reiter, 1975; Nelson and Zucker, 1987). Thus, the critical photoperiod that discerns reproductive capability is 12.5 hours of light in a day.

When adult male hamsters which have been raised in a LP laboratory environment are moved to a SP, their testes undergo regression with a complete atrophy at 6 to 10 weeks of SP regardless of the time of year at which SP exposure begins (Stetson and Watson-Whitmyre, 1986). If animals are kept in SP for an extended period, their testes recrudesce spontaneously at 20 to 25 weeks of SP (Stetson *et al.*, 1977). These animals are refractory to SP and will remain reproductively active for as long as SP is administered. This is called photorefractoriness since the animals do not show the second testicular regression under the SP. In natural habitat, the short duration of daily light in winter is expanded gradually to a photoperiod greater than 12.5 hours of light following the vernal equinox, which eventually terminates the photorefractory condition. In order to reinitiate the photosensitive state, such refractory animals need exposure of LP for 11 or more weeks in the laboratory. After such exposure, they are once again capable of undergoing gonadal regression in response to SP (Stetson *et al.*, 1977). Therefore, the effect of natural photoperiods on reproduction is precisely simulated in the laboratory by the convenient management of artificial light.

Testicular weight (alternatively, size or volume) is a good indicator of the developmental stage of spermatogenesis. There is a positive correlation between testicular weight and the developmental stages of spermatogenesis. Large testes of the photosensitive hamster on LP and the photorefractory animal on SP exhibit mature stages of spermatogenesis. Small (regressed) testes of prepubertal hamsters and hamsters exposed to

SP display far less developed stages of spermatogenesis (Gaston and Menaker, 1967). A regressed testis in SP animals shows, besides reduced mass and undeveloped spermatogenesis, a decrease in the volume of seminiferous tubule and decreases in tubular lumen diameter, interstitial space, and Leydig cell number, but not in specific gravity and Sertoli cell number when compared to an active testis of LP animals (Sinha Hikim *et al.*, 1988).

Removal of the pineal gland from the Syrian hamster prevents the photoperiodic effect of SP that causes gonadal regression in pineal intact hamsters (Stetson and Watson-Whitmyre, 1986). After induction of gonadal regression by SP exposure, pinealectomy leads to a restoration of reproductive function, similar to that observed in the pineal intact hamsters transferred from SP to LP (Matt and Stetson, 1980). Appropriately timed daily injections of melatonin to pinealectomized hamsters produce gonadal regression (Watson-Whitmyre and Stetson, 1983). Also, subcutaneous infusion of melatonin of an appropriate temporal duration to pinealectomized animals causes the testes to involute (Maywood *et al.*, 1991; Grosse *et al.*, 1993). Disruption of the neural pathway anywhere between retina and the pineal inhibits SP-induced gonadal regression in Syrian hamsters (Stetson and Watson-Whitmyre, 1976) and Siberian hamsters (Bittman *et al.*, 1991). These results thus indicate that the pineal gland, through melatonin, mediates the photoperiodic effect on reproduction.

Daily administration of melatonin in the evening, but not in the morning to intact hamsters housed in LP, causes the gonads to involute. To examine the specific sensitive time period to melatonin, adult male hamsters kept in LP were injected with melatonin at different times on daily basis (Fig. 5). Small testes were observed in the hamsters receiving injections from 5 hours prior, to 1 hour after lights off and 1 hour before lights on (Stetson and Tay, 1983). Injection of melatonin at any other time of the day had no effect on testes function. The results indicate that in pineal intact animals there are two periods where melatonin injections will cause testicular regression. A daily single injection of melatonin in

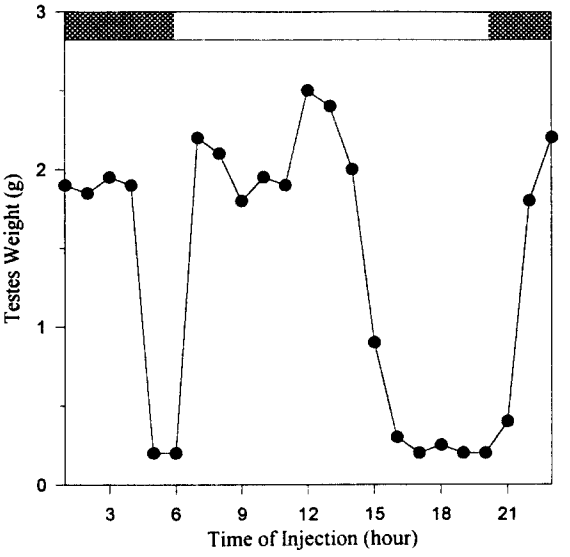

Fig. 5. Melatonin sensitivity of Syrian hamsters throughout the day. Minimum testes weight of each group injected by melatonin is shown. The photoperiod (14 hour light:10 hour dark) is presented above the curve: hours of light, open bar; hours of dark, solid bar (Stetson and Tay, 1983).

pinealectomized animals does not cause testicular atrophy (Watson-Whitmyre and Stetson, 1983). But, two or three injections of melatonin daily induce gonadal regression (Watson-Whitmyre and Stetson, 1983). These results suggest that melatonin can exert an anti-gonadotropic action.

Early studies demonstrated that constant release implants of melatonin prevent gonadal regression in SP exposed Syrian hamsters (Hoffmann, 1974). But, in contrast, similar melatonin implants induced testicular regression in LP housed hamsters (Turek, 1977). Melatonin implants have been shown to produce opposite results, depending on photoperiod (Turek *et al.*, 1975). Melatonin-containing capsules implanted prior to exposure to SP prevented testicular regression, while the same implants caused testicular regression in intact hamsters maintained in LP. The results suggest that continuously high levels of melatonin deriving from the implants act as an inhibitor of photoperiodic stimulation of reproductive activity and as an inhibitor of photoperiodic inhibition of neuroendocrine-gonadal activity (Turek *et al.*, 1975). Also, when

melatonin is given by injection in the evening to the animals with melatonin implants, testicular activity is preserved (Vaughan *et al.*, 1986). The data suggest that melatonin can exert progonadotropic action. Thus, the effect of melatonin on reproductive activity of the Syrian hamster is dependent upon the concentrations and the means of administration, the photoperiod, and animal's reproductive conditions.

At present, it is not possible to generalize how melatonin acts in even several hamster species. But, a few hypotheses have arisen on the basis of the diurnal rhythmic secretory pattern of melatonin. Pineal melatonin concentrations vary rhythmically throughout the day with peak levels occurring during the dark period (Rollag *et al.*, 1980a; Rollag and Stetson, 1981). The antigonadotropic action of melatonin has led some investigators to propose the so-called "coincidence hypothesis," which simply states that there are two rhythms that determine the reproductive response to melatonin; one is the endogenous diurnal rhythm of melatonin, and another is a rhythm of sensitivity to melatonin (melatonin-sensitive window). Only when elevated levels of melatonin coincide with the melatonin-sensitive window do animals respond as a SP signal. In LP pineal-intact Syrian, Turkish, and Siberian hamsters, the windows appear to occur at two points throughout the day; one broad window at the light:dark transition, and another narrow window just before lights are turned on (Syrian hamster: Stetson and Tay, 1983; Turkish hamster: Hong and Stetson, 1987; Siberian hamster: Stetson *et al.*, 1986). According to this model, the elevated melatonin in the animals transferred from LP to SP is assumed to overlap the melatonin-sensitive window which results in the suppression of reproductive activity. The window in pinealectomized Syrian hamsters on LP appears to be shifted and is limited to the first few hours after the light out (Stetson and Watson-Whitmyre, 1986). In pinealectomized Syrian hamsters on SP, the sensitivity to daily melatonin injections is observed throughout the first half of the dark phase (the first 6 hours), as compared to injections administered at other times (Stetson and Watson-Whitmyre, 1986). Thus, the melatonin-sensitive windows are not rigidly fixed, but appear to be dependent on photoperiod and the presence and absence of endogenous melatonin rhythms.

Another explanation of how melatonin acts is the "duration hypothesis" where the animals' reproductive activity is simply determined by the duration of the elevated melatonin peak. In this model, the exogenous melatonin administered by injection to LP animals is thought to lengthen the duration of the endogenous melatonin peak, thus mimicking a long night of SP. This idea has been supported mainly by the infusion of melatonin in pinealectomized Siberian hamsters and sheep (Bittman *et al.*, 1983; Elliott *et al.*, 1989). A melatonin infusion in pinealectomized animals, similar to the period of the melatonin peak in SP animals, elicits gonadal regression, while an infusion similar to the melatonin peak in LP animals has no effect. The longer infusion is effective in eliciting gonadal atrophy without regard to the phase of the infusion with respect to the light-dark cycle. In the Syrian hamster, "SP" melatonin infusions also induce the involution of gonads (Maywood *et al.*, 1990). On the other hand, gonadal function is maintained by the continuous administration of melatonin via implants (Maywood *et al.*, 1991). This emphasizes the importance of the daily melatonin rhythm in regulating reproduction. Even though there is a great deal of evidence that melatonin modulates the photoperiodic effect on reproduction, the site(s) on which melatonin exerts its action in seasonally breeding rodents is(are) still open.

Action sites of melatonin

A specific area of hypothalamus has been main subject to identify the action site of melatonin (Morgan *et al.*, 1994). Lesions of the SCN block gonadal regression in hamsters exposed to SP (Bittman *et al.*, 1991). These data can be interpreted as the lesions of the SCN disrupt the retinal-pineal neural pathway just as blinding does, and thus interfere with appropriate nocturnal melatonin secretion. This explanation is supported by the findings that SCN lesions eliminate the circadian rhythm of pineal serotonin N-acetyltransferase activity in rat (Roseboom *et al.*, 1996) and of melatonin production normally rising

at night. Lesions of SCN fail to prevent reproductive responsiveness to some types of exogenous melatonin administration (Maywood *et al.*, 1990); infusion of melatonin elicits gonadal regression in Syrian hamsters even after SCN ablation. But in Siberian hamsters, lesions of the SCN prevent the effects of subcutaneous melatonin infusion to elicit short day-like gonadal regression (Bittman *et al.*, 1991). On the other hand, lesions of the anterior hypothalamic region of Syrian hamsters did prevent the effects of melatonin on the reproductive system (Maywood *et al.*, 1990). Melatonin implants most effectively elicited anti-gonadotropic action when administered in or near the medial hypothalamus (Hastings *et al.*, 1988). In the white-footed mouse, melatonin pellets implanted in the SCN and retrochiasmatic region were effective in inducing a short day-like response of the reproductive axis, whereas implants in the posterior hypothalamus and midbrain were ineffective (Glass and Lynch, 1982). Chronic melatonin implants into anterior hypothalamus, preoptic area, or medial hypothalamus, but not when administered to the amygdala, midbrain, or lateral hypothalamus prevented testicular regression in short-day housed Syrian hamsters (Hastings *et al.*, 1988). The effects of brain implants and systemic infusion of melatonin can be blocked by discrete lesions of the anterior hypothalamic preoptic area and, in some but not all species, the suprachiasmatic nuclei (Bartness *et al.*, 1991). A study using brain implants in the seasonal Soay ram showed melatonin to be most effective when placed in the medial basal hypothalamus (Lincoln and Maeda, 1992). Thus there are several possible action sites of melatonin in the hypothalamus to affect reproduction, although different species have different action sites.

Since the discovery that the indole nucleus of melatonin can be iodinated (Vakkuri *et al.*, 1984), 2-[^{125}I] iodomelatonin (iodoMEL) has been used intensively as a ligand to localize melatonin-binding sites, believed to represent melatonin receptors. Earlier investigations utilizing [^{3}H] melatonin indicate that melatonin bindings were found in the brain and a variety of peripheral tissues (Cardinali *et al.*, 1979). The results were not supported by subsequent investigations (Williams and Helliwell, 1993). Primary localization of specific 2-[^{125}I] iodoMEL binding was found in the hypothalamus of the rat where binding was restricted to the SCN and the median eminence (Tenn and Niles, 1993), particularly concentrated in the pars tuberalis of the anterior pituitary (Williams *et al.*, 1989; Gauer *et al.*, 1993). In photoperiodically sensitive rodents such as hamsters and the white-footed mouse, numerous localizations of melatonin binding sites are observed mainly within the hypothalamus and the limbic system of the brain (Weaver *et al.*, 1989; Williams *et al.*, 1989). In sheep, apart from a high concentration of 2-[^{125}I] iodoMEL in the pars tuberalis of the pituitary (Morgan *et al.*, 1989), there is an extensive but less intense labeling in widespread regions of the brain (Helliwell and Williams, 1992). In the ferret, which is a seasonally breeding and highly photoperiodically sensitive animal, no binding sites for 2-[^{125}I] iodoMEL are apparent in the brain, but specific bindings appear to be restricted to the pars tuberalis and pars distalis of the pituitary (Weaver and Reppert, 1990). Therefore, the binding sites of melatonin are concentrated in species specific localities, with just two areas of binding common to most species studied, the hypothalamus and the pars tuberalis of the anterior pituitary.

As described earlier, the administration of melatonin by daily injections in the evening, but not in the morning induces gonadal regression in male hamsters (Stetson and Tay, 1983). Melatonin binding was examined in membrane fractions of hamster and rat brains in the morning and evening. There were diurnally-related changes in the number of melatonin receptor sites without changes in the affinity of the receptors; binding was greater in the evening than in the morning (Vacas and Cardinali, 1979). In contrast, the high density of melatonin binding in the rat SCN was demonstrated in the late dark period and early light period compared to other time periods (Laitinen *et al.*, 1989). Melatonin binding in the rat SCN and pars tuberalis was displayed an opposite rhythm to serum melatonin, with a peak in the late light phase and a trough in the dark phase (Gauer *et al.*, 1993). Both high and low

affinity of melatonin binding are shown with respect to the light-dark cycle. In hamster brain synaptosomal membranes, 2-[^{125}I] iodoMEL binding yielded both high and low affinity components (Niles *et al.*, 1987). There is still no consistency of diurnal rhythms of melatonin receptor numbers and the affinity change.

At the cellular level, melatonin attenuates forskolin-stimulated cyclic adenosine monophosphate (cAMP) and activation of cAMP-dependent protein kinase in primary cell cultures of ovine pars tuberalis (Morgan *et al.*, 1990; Hazlerigg *et al.*, 1991). In addition, prolonged exposure to melatonin sensitizes the adenylate cyclase transduction pathway such that basal and forskolin-activated production of cAMP is increased following removal of melatonin (Hazlerigg *et al.*, 1993). The findings indicate that melatonin receptors are linked to a G-protein. Although a number of data suggest putative action sites of melatonin, the functional aspect remains to be investigated.

Melatonin has been implicated in ultra feedback of luteinizing hormone (LH) at the median eminence of adult rats to control the release of GnRH (Nakazawa *et al.*, 1991). Melatonin is capable of increasing in vitro GnRH release from median eminence and pars tuberalis explants, but decreasing in vitro LH release from the same subjects. The results suggest that melatonin could be involved in the regulation of LH release by controlling the release of GnRH.

Recently, the melatonin receptor was cloned by expression cloning from *Xenopus laevis* dermal melanophores (Ebisawa *et al.*, 1994). Subsequently, other types of melatonin receptors were identified in human retina and brain and chicken brain (Reppert *et al.*, 1994, 1995). The receptors are members of G protein-coupled receptor superfamily with high percentage of amino acid homology with each other.

Neuroendocrine role of melatonin on reproduction

Gonadal regression caused by exposure to SP or melatonin treatment is accompanied by a marked decrease in pituitary and plasma follicle-stimulating hormone (FSH), LH, and prolactin (PRL) which precede the reduction of testes weight (Fig. 6, Steger *et al.*, 1985) and the number of LH, FSH, and PRL receptors in the testes (Bartke *et al.*, 1987; Klemcke *et al.*, 1987). During testicular recrudescence, serum levels of reproductive anterior pituitary hormones return to those characteristic of LP animals. In SP hamsters, the reduction in the concentrations of serum gonadotropins appears to be due to the suppression of hypothalamic GnRH release. Treatment of regressed animals with GnRH increases serum LH levels to those of LP animals treated with GnRH (Pickard and Silverman, 1979). In *in vitro* culture of the anterior pituitary, GnRH treatment (given at one hour intervals) augments LH and FSH in a similar way in both LP and SP animals with regressed testes (Jetton *et al.*, 1991). There is also direct evidence that hypothalamic GnRH content is significantly increased in hamsters with involuted gonads, and then decreases to amounts characteristic of LP animals during recrudescence (Urbanski *et al.*, 1991). A plausible explanation for the findings mentioned above is that inhibitory photoperiods may result in attenuated or altered GnRH production and/or secretion. However, at present no data from the hamster are available on hypothalamic GnRH secretory patterns in either LP or SP animals. The effect of inhibitory

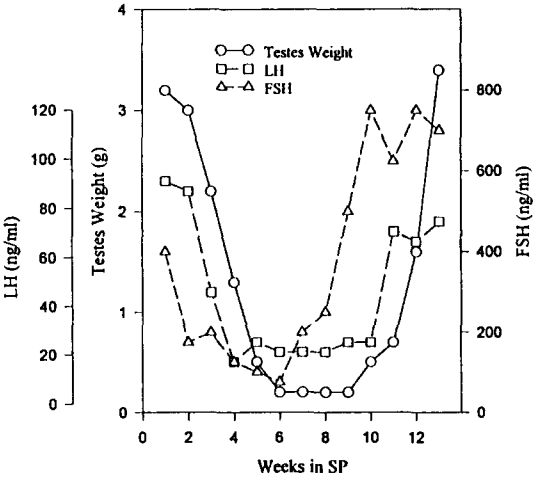

Fig. 6. Effect of short photoperiod (6 hour light:18 hour dark) on testicular weight, LH, and FSH in male hamsters. Standard deviations are omitted for ease of understanding (Choi, 1994).

photoperiod in the hamster (SP) and the sheep (LP) is to produce a decrease in LH pulse frequency, leading to low mean LH and FSH values (Scaramuzzi and Baird, 1977). Properly timed melatonin treatments mimic the photoperiodic effect on reproduction (Stetson and Tay, 1983). Thus, the results suggest that the proper melatonin administration affects the production and/or release of GnRH at hypothalamic level.

GnRH is released in a pulsatile manner from the GnRH neuronal terminals in the median eminence. The peptide is stored in the nerve terminals prior to release into hypophysial portal blood vessels. Gonadotropins are secreted episodically in response to the GnRH (Levine and Duffy, 1988). The activity of GnRH neurons is influenced via feedback loops by gonadal steroid hormones as well as brain neurotransmitters. Testosterone (T) regulates GnRH release from the hypothalamus both *in vivo* and *in vitro* (Levine and Duffy, 1988; Park *et al.*, 1988). Also, the observation that changes in prohormone levels fluctuate in an inverse relation to changes in GnRH levels, suggests the involvement of T in the processing of precursor pro-GnRH mRNA to GnRH (Roselli *et al.*, 1990). SP exposure is thought to increase the sensitivity of the hypothalamus to steroid feedback since a small concentration of T in reproductively inactive males effectively maintains the low release of pituitary hormones (Sisk and Turek, 1982). But, elimination of T by castration of animals with regressed testes is unable to alter gonadotropin levels (Tate-Ostroff and Stetson, 1981). The latter observations challenge the steroid-dependent decrease of serum gonadotropins in SP animals and suggest that T may not be directly involved in the suppression of gonadotropin secretion in photoinhibited animals. Moreover, the observation that GnRH neurons do not retain steroids to any appreciable extent suggests that the regulatory effect of steroid hormones may not be exerted directly on GnRH neurons, but rather may be mediated by other neurons in the vicinity of the GnRH neurons (Huang and Harlan, 1993).

Pinealectomy, which preserves functional testes in the Syrian hamster, regardless of photoperiod, is associated with undiminished levels of LH (Roberts *et al.*, 1985a), whereas pineal-intact animals on SP display a decrease of gonadotropins. In light of the fact that serum gonadotropins reflect the release of GnRH, the suppression of GnRH release by SP appears to be achieved by the mediation of melatonin in the neuroendocrine system regulating reproduction in the Syrian hamster. There is no report of melatonin binding on GnRH neurons. Therefore, it is likely that melatonin somehow affects the GnRH neurons by acting on other neurons in the brain.

Opioidergic neurons are strong candidates because of their inhibitory action on GnRH release. Some data indicate that SP or melatonin treatment results in an alteration of hypothalamic opioid contents (Roberts *et al.*, 1985b; Juss *et al.*, 1991). Moreover, the finding that opioidergic neurons synapse with GnRH neurons (Chen *et al.*, 1989) suggests that opioidergic neurons mediate the photoperiodic (or melatonin) message to the GnRH neuron to control reproductive activity.

It has been generally accepted that opioids are involved in the control of hormone release from the pituitary in many mammals. Opioid agonists are inhibitory to FSH and LH but stimulatory to PRL, with an opposite effect of opioid antagonists (Bicknell, 1985). Injection of naloxone, an opioid receptor antagonist, causes a prompt increase in secretion of LH in sexually active male hamsters, but this effect is reduced or absent in males that are sexually inactive due to exposure to SP (Roberts *et al.*, 1985a; Choi, 1994). In addition, naloxone administration induces an increase in LH levels throughout the day in males kept in LP, but it has no effect on LH concentrations that have been decreased by SP (Roberts *et al.*, 1985b; Choi, 1994). The results indicate either that opioidergic neurons, in affecting LH release, are functioning in LP animals, but not in SP animals, or that the sensitivity of opioid target tissue changes with photoperiod. Naloxone has no effect on serum LH levels in castrated hamsters regardless of photoperiod (Roberts et al., 1985a; Choi and Stetson, 1993). However, serum LH concentrations are elevated to castration levels by naloxone in castrated hamsters on LP in which LH

levels were suppressed by T implants. This stimulatory effect of naloxone on LH is not detected in castrated SP animals treated with T, suggesting that the treatment of reproductively inactive hamsters with gonadal steroids does not reinstate the LH response to naloxone (Choi and Stetson, 1993). Therefore, the absence of naloxone's effect observed in SP animals is not simply due to a change in steroid secretion (Roberts *et al.*, 1985a).

In a study to assess the effect of photoperiod on the LH response to naloxone, Eskes *et al.* (1984) have reported that the stimulatory effect of naloxone remains at day 34 of SP exposure but not at day 42. When male hamsters with regressed gonads were transferred to LP, naloxone had no effect on day 21 of LP exposure but increased LH levels on day 62 (Eskes *et al.*, 1984, Choi, 1994). The similar results were also observed in the animals treated with melatonin in the evening, but not in the morning (Fig. 7). The findings indicate that the opioid system becomes functional during testicular recrudescence by the exposure to LP or to the prolonged administration of melatonin.

In addition to the acute effects of naloxone treatment on the gonadotropins, hamsters were chronically treated with naloxone to determine if long-term blockade of the opioid system exposed to SP would induce a reproductive response (Chen *et al.*, 1984). A single daily injection of naloxone to male hamsters has been shown to reverse, in part, the inhibitory effect of SP on testicular size. Thus, it is possible that the photoperiodic effects on gonadal activity in male hamsters may be mediated via physiological alteration of the opioid system.

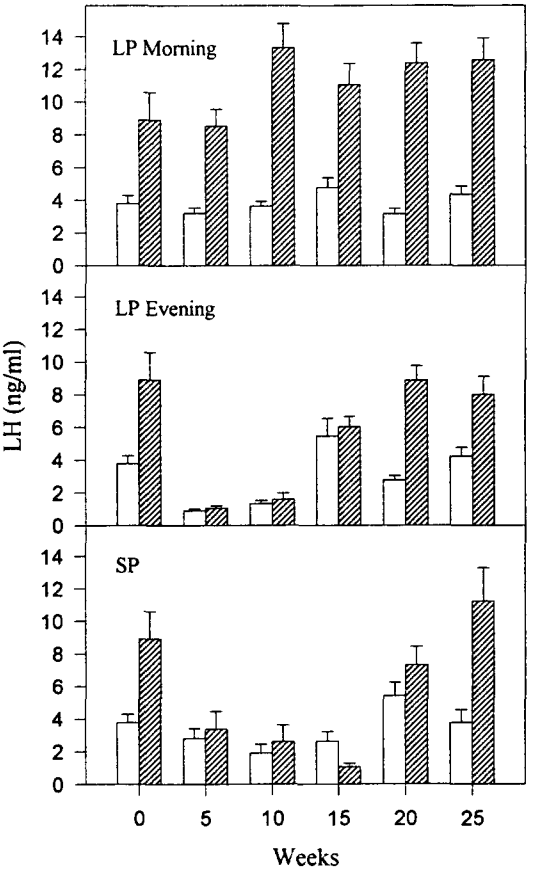

Fig. 7. Effect of naloxone on LH secretion in hamsters injected with melatonin in the morning or evening. Animals housed in LP were injected with melatonin in the morning (LP morning) or evening (LP evening). Another group of animals was moved to SP and injected with saline. Blood samples were collected 15 minutes following the injections of saline (open bar) or naloxone (closed bar) (Choi, 1994).

Results

The neuroendocrine mechanism by which melatonin exerts its effect in the control of mammalian reproduction is largely unknown. It is hampered by the lack of acute influence of melatonin on the reproductive hormone LH, FSH, and PRL. However, the finding that augmented hypothalamic GnRH content in the gonadally regressed animal treated with short photoperiod or timed melatonin administration may imply some possible causal relationship between GnRH neuron and melatonin. Even though the binding site of melatonin has been demonstrated in the hypothalamic area in which GnRH neuron are also distributed, the main neuronal pathway that the melatonin affects remains to be investigated. One of the prime candidates is the opioid neuron whose activity is affected by the long term treatment of photoperiod and melatonin and whose influence is on the release of the

reproductive hormones through the GnRH neuron. But it can not be ruled out other intervening neuronal system mediating the known effect of melatonin.

The novel isolation and characterization of melatonin receptors in the brain and retina (Ebisawa *et al.*, 1994; Reppert *et al.*, 1994, 1995) will facilitate to identify its target sites in the regulation of reproduction as well as in other functional properties. It also will serve to investigate signal transduction mechanism of melatonin to its receptor in responsive cells. The study at the molecular level will facilitate to test the analogues of melatonin for the pharmacological purpose. Therefore, the pineal gland through the action of melatonin on the receptor is now realized to have substantial effects at the cellular and molecular levels.

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송과선 호르몬 멜라토닌의 생식 생리학

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멜라토닌은 대뇌와 소뇌 사이에 위치한 송과선에서 분비되는 호르몬으로 빛이 없는 밤에만 분비된다. 멜라토닌은 분자적 수준에서부터 개체의 행동에 이르기까지 다양한 기능을 보인다. 특히, 생식에 미치는 영향은 광범위하여, 온대지방에 사는 대부분의 동물은 주위 환경에 적응하여 종족을 유지하는 유일한 계절적 번식을 한다. 햄스터의 생식활동은 여름에 왕성하고 겨울에 정지된다. 이는 많은 환경요소중 광주기의 효과로 입증되었다. 반면 송과선을 제거하면 광주기의 영향은 사라진다. 즉 생식에 미치는 광주기의 효과가 송과선에 의해 중재됨을 의미한다. 또한 송과선 호르몬인 멜라토닌의 적절한 처리는 생식활동을 억제한다. 따라서, 멜라토닌은 생식에 미치는 광주기의 정보를 생식내분비계로 전달하는 신경전달물질로 사료된다. 시상하부의 특정부위를 절제한 후 광주기나 멜라토닌을 처리하여 멜라토닌의 작용부위에 관한 연구가 되었으나 동물마다 차이점을 보인다. 대부분의 동물에서 공통적인 부위는 suprachiasmatic nuclei와 pars tuberalis이다. 멜라토닌이 생식에 미치는 작용기작은 아직 밝혀지지 않았다. 이는 멜라토닌이 여포자극호르몬과 황체호르몬에 대한 단기적 효과의 부재에 기인한다. 그러나 적절한 멜라토닌의 장기적처리는 이들 호르몬의 분비를 저하시키고, 시상하부에서의 gonadotropin-releasing hormone (GnRH) 양을 증가시킨다. 이 결과는 멜라토닌의 지속적 처리가 시상하부로부터의 GnRH 분비를 감소시킴으로써 생식활동을 억제하는 것으로 사료된다. 그러나, 멜라토닌에서 GnRH 신경까지의 정보전달은 아직 밝혀지지 않았다. Opioid 신경에 대한 광주기와 멜라토닌의 효과가 동일한 점은 opioid 신경의 매개체 역활을 제시하고 있다. 최근에 멜라토닌 수용체가 개구리의 피부와 몇몇 동물의 뇌와 시세포에서 크로닝되었다. 이 수용체는 G protein과 관련되고 cAMP 생성을 억제한다. 앞으로 이 멜라토닌 수용체의 존재여부와 분자생물학적 연구는 멜라토닌의 작용부위와 표적세포에서의 작용기작을 설명하는데 크게 기여할 것으로 기대된다.