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〈종 설〉

Influence of Melatonin on Reproductive Function in Male Golden Hamsters

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수컷 골든 햄스터의 생식기능에 미치는 멜라토닌의 영향

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ABSTRACT : Golden hamsters show the reproductive activity that is determined by the photoperiod (length of light per day). Photoperiod is an environmental factor that is predictable through an entire year. The hamsters are sexually active in summer during which day length exceeds night time. The critical length is at least 12.5 hours of light in a day where reproductive function is maintained. The information of photoperiod is mediated by the pineal gland because removal of pineal gland blocks the influence of photoperiod on reproductive activity. The hamsters without pineal gland maintain sexual activity and promote it in a situation that suppresses gonadal activity. The pineal gland secretes melatonin that reflects the photoperiod. The appropriate administrations of melatonin into both pineal intact and pinealectomized hamsters lead to a gonadal regression. The results suggest that melatonin constitutes a part of control mechanism whereby environmental information is transduced to neuroendocrine signal responsible for the functional integrity of the reproductive system. Despite of the intense studies, the action site of melatonin is on the whole unknown. It is mainly due to the lack of acute effect of melatonin on the secretion of reproductive hormones. However, sexually regressed animals display the low levels of gonadotropins and the augmentation of the hypothalamic gonadotropin-releasing hormone (GnRH) content, implying that the antigonadotropic effects either by photoperiod and/or by the treatment of melatonin are mediated by the GnRH neuronal system. The action mechanism by which melatonin exerts its effect on GnRH neuron needs to be investigated. Recent cloning of melatonin receptor will contribute to examine various and putative potencies of melatonin via its anatomical identification and the action mechanism of melatonin on target tissues at the molecular level.

Key words : Melatonin, Photoperiod, Reproduction, Gonadotropin-releasing hormone.

요 약 : 골든 햄스터의 생식활동은 광주기(하루 중 조명 시간)에 의해 결정된다. 광주기는 일년 동안 예측할 수 있는 환경 요인이다. 주간 길이가 야간 길이보다 긴 여름에 햄스터의 생식 활동은 왕성하다. 생식 기능을 유지시키는 조명 시간은 하루에 적어도 12.5시간이다. 송과선을 제거시키면 광주기의 정보가 억제되기 때문에, 광주기의 정보는 송과선을 통하여 중재된다. 송과선을 제거 당한 햄스터는 생식 활동이 유지되고, 생식소 기능을 억제하는 상황에서도 생식 기능을 촉진시킨다. 송과선은 멜라토닌을 분비하고 멜라토닌은 광주기 정보를 반영한다. 멜라토닌을 적절히 투입하면 송과선과 무관하게 생식소 퇴화가 유도된다. 생식체계를 기능적으로 통합하는 신경내분비 신호로 환경 정보가 전환하는 기전을 멜라토닌이 조절함을 시사한다. 광범위한 연구에도 불구하고, 멜라토닌의 작용부위는 알려지지 않았다. 이는 멜라토닌이 생식 호르몬의 분비에 미치는 즉각적인 효과가 없기 때문이다. 그러나, 성적으로 퇴화된 동물들은 생식 호르몬 수준이 낮고 시상하부 내 GnRH 양이 증가한다. 광주기 혹은 멜라토닌 처리가 생식 기능을 억제하는 효과는 GnRH 신경계에 의해 중재됨을 의미한다. 멜라토닌이 GnRH 신경에 미치는 작용 기전이 조사되어야 한다. 멜라토닌 수용체가 클로닝되어, 목적 조직에 미치는 멜라토닌의 작용 기전과 해부학적 위치를 통하여 멜라토닌의 다양하고 잠재적인 능력을 분자수준에서 연구하는데 공헌할 것이다.

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INTRODUCTION

It has long been recognized that most animals in a temperate zone show a limited period of time in parturition. Golden hamsters give rise to births in a photoperiod that is greater than 12.5 hours of a day. The reproductive activity of the animals is suppressed when the photoperiod is equal to or less than 12 hours per day, regardless of sex. The animals demonstrate a rhythmic fluctuation of sexual activity throughout a year, alternating active and inactive stages. The annual cycle of reproductive capacity is conveniently categorized into four phases(Stetson & Watson-Whitmyre, 1984): the photosensitive phase, regressing phase, regressed phase, and photorefractory phase. Under natural conditions in a temperate latitude, hamsters are sexually active during spring (photorefractory phase) and summer until the autumnal equinox (photosensitive phase) in mid-late September. In the fall, hamsters experience gonadal regression (regressing phase) as the photoperiod shortens. In addition, since the hamsters are known to be hibernators during the winter season, spending great amount of time in their subterranean habitat, they are exposed to gradually fewer and fewer hours of daylight. This results in gonadal atrophy (regressed phase). In late winter, the gonad begins to recrudescence, but not as a consequence of increasing daylength. Exposure of animals to constant darkness at this time of the year does not suppress or delay gonadal recrudescence. Thus the neuroendocrine-gonadal axis is referred to as refractory to photoperiod. As a results, hamsters are capable of reproducing immediately when they emerge from their hihernaculum. This annual cycle of reproduction increases the likelihood of survival.

PHOTOPERIOD AND REPRODUCTION

The seasonal change in the reproductive status of the hamster is copied in the laboratory during any season of the year with carefully controlled lighting adjustment. When the adult animals are exposed to long photoperiod (LP, $\geq$12.5 hours of light per day) including constant light, they maintain the reproductive activity(Elliott, 1976). On the other hand, short photoperiod (SP, $\leq$12 hours of light per day) including constant darkness causes the gonads to regress. Light deprivation by enucleation leads to gonadal regression. Exposure of an animal with regressed gonads to photoperiod greater than or equal to 12.5 hours of light causes gonadal regrowth. Thus the critical photoperiod that discerns reproductive capability is 12.5 hours of light in a day.

The findings that has been known up to date are reviewed here within the confines of adult male golden hamster in regulating reproductive activity. Testicular weight (alternatively, volume or size) is a convenient indicator of the developmental stage of spermatogenesis. There is a positive correlation between testicular weight and the developmental stages of spermatogenesis. Large (relatively heavier) testes of the photosensitive hamster on LP and the photorefractory animal on SP exhibit mature stages of spermatogenesis. Small (regressed, relatively lighter) testes of prepubertal and adult hamsters exposed to SP display far less developed stages of spermatogenesis(Gaston & Menaker, 1967). A regressed testis in SP animals shows, besides reduced mass and undeveloped spermatogenesis, a decrease 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).

When adult male golden 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. If animals are kept in SP for an extended period, their testes regain sexual activity spontaneously at 20 to 25 weeks of SP. These animals are refractory to SP and will remain reproductively active as long as SP is administered. The animals do not show the second testicular regression under the SP, which is named photorefractory phase. 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 at least 11 weeks in the laboratory. Following such exposure, they are once again capable of undergoing gonadal regression in response to SP.

PINEAL GLAND AND REPRODUCTION

The photoperiodic influence of SP that causes gonadal

regression in pineal intact hamsters is abolished by surgical removal of pineal gland from the golden hamster(Stetson & Watson-Whitmyre, 1984, 1986). When the sexually mature hamsters are pinealectomized and transferred to a SP, they do not respond to the SP but maintain active sexual activity. 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. The results thus indicate that the pineal gland mediates the photoperiodic effect on reproduction. A major hormone that the pineal gland secretes is melatonin. Appropriate administrations of melatonin to hamsters causes the testes to involute.

MELATONIN SYNTHESIS AND PHOTOPERIOD

Melatonin (N-acetyl-5-methoxytryptamine) was isolated and characterized from beef pineal gland four decades ago(Lerner et al., 1958). It is a product of tryptophan metabolism by the pineal gland. The pineal gland (epiphysis cerebri) is believed to uptake tryptophan into the pinealocyte by a neural amino acid transport mechanism. 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-methyltransferase 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). It is believed that melatonin synthesized in the pineal gland is 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 gland and other brain areas in mammals examined so far(Maywood et al., 1991).

In all mammals examined, the duration of elevated melatonin in both pineal gland and serum is proportional to the length of dark period(Stetson & Watson-Whitmyre, 1986). When the animals are moved from LP to SP, the pineal gland melatonin content expands gradually(Hastings et al., 1987). Even in a constant darkness, the animals show a similar pattern of melatonin production to that 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 synthesis and causes a rapid decline in tissue and blood levels of the hormone. These findings indicate that melatonin production within the pineal gland is regulated by the light.

The photic signal reaches the pineal gland by a multi-step pathway. The light message is perceived by the ocular photoreceptors that send signal to the suprachiasmatic nucleus (SCN) via the retinohypothalamic track. The SCN sends neural projections to paraventricular nucleus (PVN) of the hypothalamus. From the PVN, the information travels to the spinal cord through brain stem and exits via the thoracic preganglionic sympathetics those synapse on postganglionic fibers in the superior cervical ganglion (SCG). Postsympathetic neurons (nervi conarii) projecting from the SCG innervate the pineal gland. All of the neurotransmitters involved in this long multisynaptic pathway from the retina to pineal gland have not yet been identified, though some are known. One of the factors that affect the synthesis of melatonin is norepinephrine released from the axonal terminal of the postsympathetic neurons present within the pineal gland(Dodge & Badura, 2001). Disruption of the neural pathway at any point between the retina and the pineal alters the pattern of melatonin production. Thus, the pineal gland is considered to be a neuroendocrine transducer, as the neural input to this organ is converted into an endocrine output.

EFFECTS OF MELATONIN ON REPRODUCTIVE FUNCTION

Three ways of melatonin administration, injections, implants, and infusion, have been applied. Firstly, melatonin was injected at different times on daily basis into pineal intact mature male hamsters kept in LP (14 hour light:10 hour dark, 14L:10D). Regressed testes were observed in the animals receiving injection from 5 hours prior to 1 hour after light off and 1 hour before lights on(Stetson & Tay, 1983). Injection of melatonin at other time of the day had no effect on testes function. The results indicate that, in pineal gland intact hamsters, there are two periods where melatonin injection might cause testicular involution. But a daily single injection into pinealectomized animals was not shown to cause testicular atrophy(Watson-Whi-

tmyre & Stetson, 1983). However, two or three injections of melatonin daily induce a gonadal regression. The results suggest that melatonin could exert an antigonadotropic action. Secondly, implants (capsules that contain melatonin) that releases melatonin constantly were applied. When hamsters with the implants were exposed to SP, gonadal regression was prevented. But similar melatonin implants induced testicular regression when animals were housed in LP. Thus melatonin implants showed to produce opposite outcome, depending on the photoperiod(Turek et al., 1975). The results have not been understood yet. When the animals were treated with a combination of injection in the evening and melatonin implants, testicular function was preserved. The results suggest that melatonin could exert progonadotropic action. It may be due that high levels of melatonin released from the implants might mask the rhythmic properties of melatonin. Thus the effect of melatonin is dependent on the concentration, the mean of administration, photoperiod, and the reproductive condition of the animal. Lastly, infusion was applied to mimic the endogenous production of melatonin. It uses a tool that operates to administer melatonin for a given period of time in a day. Therefore, the levels of melatonin measured in either LP or SP can be exactly manipulated. The longer infusion resembles SP and the shorter infusion does LP. In golden hamsters, the critical infusion period of melatonin in a day that maintain reproductive activity is known as 6 hour. The longer melatonin infusion into the pinealectomized animals elicited the involution of gonads without regard to the phase of the infusion with respect to the light-dark cycle(Maywood et al., 1990). On the other hand, testicular function is maintained by the continuous administration of melatonin via implants(Maywood et al., 1991). This again emphasizes the importance of the daily melatonin rhythm in regulating reproduction.

PHASE AND DURATION HYPOTHESES

It is at present not possible to generalize how melatonin acts. But, a few hypotheses have been arisen with the results observed from other several hamster species and other seasonal breeders on the basis of the diurnal rhythmic secretory pattern of melatonin. Melatonin concentrations vary rhythmically throughout the day with peak level occurring during the dark period (Rollag & 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 the rhythm of sensitivity to melatonin (melatonin-sensitive window). Only when elevated levels of melatonin coincide with the melatonin-sensitive window, animals can respond to the SP signal. On LP, the windows of pineal-intact golden, Turkish, and Siberian hamsters 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(Stetson & Tay, 1983; Hong & Stetson, 1987; Stetson et al., 1986). According to the model, the elevated levels of melatonin in the animals transferred from LP to SP are assumed to overlap the melatonin -sensitive window which results in the suppression of reproductive activity. The window in pinealectomized golden hamsters on LP appears to be shifted and limited to the first few hours after the light out(Stetson & Watson-Whitmyre, 1986). In pinealectomized golden 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 & 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 reproductive activity of the animals 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 experiments where the infusion of melatonin was done in pinealectomized Siberian hamster and sheep(Bittman et al., 1983; Elliott et al., 1989; Powers et al., 1997). A melatonin infusion in pinealectomized animals, similar to the period of melatonin peak in SP animals, elicits gonad regression, while an infusion similar to the melatonin peak in LP animals had no effect.

Taken together, the two hypotheses put the response of animals to photoperiod on the daily melatonin rhythm in the regulation of reproduction.

Despite of a number of evidences that melatonin manipulates

the photoperiodic influence on reproduction, the sites on which melatonin exerts its action in seasonally breeding animals are however, not clarified yet.

ACTION SITES OF MELATONIN

A specific area of hypothalamus has been the main subject to identify the action site of melatonin(Morgan et al., 1994; Freeman & Zucker, 2001). Lesions of anterior hypothalamic area of golden hamsters prevented the effects of melatonin on the reproductive system(Maywood et al., 1990). When melatonin implants were administered in or near the medial hypothalamus, it elicited antigonadotropic action effectively(Hastings et al., 1988). More detailed studies showed that chronic melatonin implants prevented testicular regression in SP-housed golden hamsters when administered into anterior hypothalamus, preoptic area, or medial hypothalamus, but not when administered to the amygdala, midbrain, or lateral hypothalamus(Hastings et al., 1988). Thus there are several putative action sites of melatonin in the hypothalamus that might affect the reproduction of the animal.

Lesions of the SCN also block gonadal regression in hamsters exposed to SP(Bittman et al., 1991). The findings can be explained as the lesion of SCN disrupt the retinal-pineal neural pathway, thus interfering with appropriate nocturnal melatonin secretion. From a vast amount of results examined in photoperiodically sensitive animals using radiolabelled melatonin, the binding sites of melatonin are found as being concentrated in species-specific localities, with two binding areas common to most species studied, the hypothalamus and the pars tuberalis of the anterior pituitary. It is believed that the former is involved in the regulation of circadian rhythm and the latter in the control of reproduction.

NEUROENDOCRINE ROLE OF MELATONIN

Gonadal regression caused by either exposure to SP or melatonin treatment is accompanied by a marked reduction in pituitary and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, which precede the decrease of testes weight(Choi, 1996). During testicular recrudescence, serum levels of reproductive anterior pituitary hormones return to those characteristic of LP animals. In SP hamsters, the reduction in the concentration of serum gonadotropins appears to be due to the suppression of hypothalamic gonadotropin-releasing hormone (GnRH) release. Treatment of regressed animals with GnRH increases serum LH levels to those of LP animals treated with GnRH(Pickard & 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 as in both LP and SP animals with regressed testes(Jetton et al., 1991). There is also a direct evidence that hypothalamic GnRH content significantly increases in hamsters with involuted gonads, and then decrease to an amount characteristic of LP animals during recrudescence(Urbanski et al., 1991). A possible explanation for the findings is that inhibitory photoperiods may result in attenuated or altered GnRH production and/or release, without affecting the number and distribution of GnRH neuron(Horton & Yellon, 2001). However, no data at present is available with regard to the hypothalamic GnRH secretory pattern in either LP or SP hamsters. An indirect observation is that the pulse frequency of LH measured in serum was abolished, if any remarkably decreased(Swann & Turek, 1988). Thus it is reasonable to note that treatments that suppress reproductive activity may affect the synthesis and/or release of GnRH at hypothalamic levels.

Pinealectomy, which preserves functional testes in the golden hamsters regardless of photoperiod, is associated with undiminished levels of LH(Roberts et al., 1985), 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 golden hamsters. There is no observation of melatonin binding on GnRH neurons so far. Therefore, it is likely that melatonin somehow affects the GnRH neurons by acting on other intervening neurons.

One of the candidates as the intervening neurons is the opioidergic neuron(Choi, 1996). It morphologically synapses with GnRH neurons(Chen et al., 1984). The treatment of naloxone, an opioid receptor antagonist, causes a prompt increase in the secretion of LH in sexually active hamsters. However, this effect is reduced or absent in males that are sexually inactive due to exposure to SP(Roberts et al., 1985; Choi, 1994). In

addition, naloxone administration induces an increase in LH levels throughout the day in males in LP, but it has no effects on LH concentration that have been decreased by SP. The results indicate that opioidergic neurons affecting LH release functions in LP animals, but not in SP animals. Apart from the acute effect of naloxone on the gonadotropins, other effect of naloxone was also examined in hamsters by chronic treatment to determine if long-term blockade of the opioid system exposed to SP would induce a reproductive response. 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 photoperiodic effect on gonadal activity in males may be mediated via physiological alteration of the opioid system. However, whether the alteration of opioid system causes the effect of inhibitory photoperiod or is a concurrent phenomenon remains to be examined.

MELATONIN RECEPTOR

The melatonin receptor was cloned by expression cloning from Xenopus laevis dermal melanophores(Ebisawa et al., 1994). Subsequently, other types of melatonin receptor were identified from various tissues of many animals(Reppert et al., 1994, 1995; Weaver et al., 1996). The receptors are a member of G protein -coupled receptor superfamily with high percentage of amino acid homology with each other. At the cellular level, melatonin attenuates forskolin-stimulated cyclic adenosine monophosphate (cAMP) and thus 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 of the cells to melatonin sensitizes the adenylate cyclase transduction pathway such that basal- and forskolin-activated production of cAMP increases following removal of melatonin (Hazlerigg et al., 1993).

CONCLUSION

The neuroendocrine mechanism by which melatonin exerts its effect on the regulation of mammalian reproduction is largely unknown. It is hampered by the lack of acute impact of melatonin on the other reproductive hormones, LH, FSH, and prolactin. However, the findings that augmented hypothalamic GnRH content in the gonadally regressed animal 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 elucidated. The novel isolation and characterization of melatonin receptor in the brain will facilitate the identification of its target site in the regulation of reproduction as well as in other functional properties. It will also serve to examine signal transduction mechanism of melatonin to its receptor in responsive cells. The study at the molecular level will be helpful to test the analogues of melatonin for the pharmacological purpose.

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