발생과 생식 제 6권 제 1호, 45~54 (2002)
Dev. Reprod. Vol. 6, No. 1, 45~54 (2002)

A Study for the Expression of Melatonin Receptor Gene and Reproductive Indices in Golden Hamsters Exposed to Photoperiods

Donchan Choi†, Hyungjae Choi, Sinae Lim[1] and Changeun Park[2]

Division of Natural Science, Yong-In University, Yongin, Geonggi-do 449-714, Korea
[1]Genetic Toxicology Department, National Institute of Toxicological Research, KFDA, Seoul 112-824, Korea
[2]Infertility Medical Center, CHA General Hospital, Seoul 135-081, Korea

골든 햄스터에서 광주기에 따른 멜라토닌 수용체 유전자 발현과 생식 지수들에 관한 연구

최돈찬† · 최형재 · 임시내[1] · 박창은[2]

용인대학교 자연과학대학 자연과학부, [1]국립독성연구원 유전독성과, [2]차병원 여성의학연구소

ABSTRACT : Reproductive activity of golden hamsters(*Mesocricetus auratus*) is regulated by the photoperiod. They are sexually active in summer and inactive in winter. Melatonin, a pineal hormone, has been known to mediate sexual activities in seasonal breeding animals. Melatonin receptor was recently identified in several animal species including human. But little has been known about it in relation to the reproductive activities of golden hamsters. By using reverse transcription polymerase chain reaction(RT-PCR) methods, a portion of the melatonin receptor gene(309 nucleotides) was identified in golden hamsters. The nucleotide sequence of the melatonin receptor and the amino acid sequence deduced were compared to those reported in other animals. Melatonin receptors were obviously detected in hypothalamus, pituitary containing pars tuberalis, blood, and spleen. Although the testicular weights and the levels of reproductive hormones were dramatically affected by photoperiods, the expression of melatonin receptor was not markedly changed by them. These results suggest that the action of melatonin in regulating reproduction might be mainly due to the affinity of melatonin receptor rather than the density of melatonin receptor.

Key words : Melatonin receptor, Golden hamster, Photoperiods, Reproduction.

요 약 : 골든 햄스터의 생식활동은 광주기에 의해 조절된다. 그들의 생식능력은 여름에 왕성하고 겨울에는 퇴화한다. 송과선에서 분비되는 멜라토닌은 계절적 번식동물에서 생식활동을 중재한다. 멜라토닌 수용체가 최근에 사람을 포함하는 몇몇 동물에서 확인되었지만 골든 햄스터의 생식능력과 관련하여 알려진 바가 많지 않다. 역전사 PCR 방법을 사용하여 멜라토닌 수용체의 일부 유전자를 동정하였다(309 염기). 멜라토닌 수용체의 핵산 서열과 추론된 아미노산 서열을 보고된 다른 동물들과 비교하였다. 멜라토닌 수용체는 시상하부, 뇌하수체, 혈액, 지라에서 명백히 탐지되었다. 광주기가 정소 무게 및 생식 호르몬에 현저한 영향을 주었지만 멜라토닌 수용체의 발현에는 영향을 뚜렷하게 보이지 않았다. 이러한 결과는 생식을 조절하는 멜라토닌은 그 수용체의 수보다 수용체에 결합하는 친화도가 더 중요함을 시사한다.

INTRODUCTION

The sexual activity of golden hamster is active in summer and inactive in winter. These changes of reproductive activity are established to be mediated by the photoperiod(length of the light per day)(Gaston & Menaker, 1967; Elliott, 1976; Stetson & Watson-Whitmyre, 1984; 1986). Long photoperiods(LPs, ≥12.5 hour light per day) maintain and promote the reproductive activity but short photoperiods(SPs, ≤12 hour light per day) suppress it. The photoperiodic information is transmitted to the pineal gland, since it has been well documented that the removal of pineal gland abolishes the effects of photoperiods(Stetson & Watson-Whitmyre, 1984; 1986). Melatonin, which is a major hormone secreting from the pineal gland, transduces the environmental photoperiod signal to the reproductive neuroendocrine system(Stetson & Watson-Whitmyre, 1984; Steger et al., 1985;

* This work was supported by Korea Research Foundation Grant(KRF-99-003-D00170).

†교신저자: 경기도 용인시 삼가동 470, 용인대학교 자연과학대학 자연과학부. (우) 449-714, (전) 82-31-330-2781, (팩) 82-31-330-2886, E-mail: dcchoi @eve.yongin.ac.kr

Reiter, 1991a). Appropriately timed daily administrations of melatonin mimic the effect of SP(Stetson & Tay, 1983; Maywood et al., 1991). Hamsters with regressed testes either by exposure to a SP or by timed melatonin treatments exhibit remarkable reduction of testicular weight preceded by a decrease of reproductive hormones in serum such as luteinizing hormone (LH), follicle stimulating hormone(FSH), and prolactin(Jackson et al., 1984; Choi, 1996). Secretion of gonadotropins is regulated by the gonadotropin releasing hormone(GnRH) that is reached the anterior pituitary through the hypothalamo-pituitary portal blood vessel following release from the hypothalamus. Thus, the levels of gonadotropins in blood reflect the degree of GnRH. Therefore, it is reasonable to note that melatonin by proper administration somehow affects the GnRH neuron.

Recently, melatonin receptor was cloned from *Xenopus laevis* dermal melanophores(Ebisawa et al., 1994). Subsequently, other types of melatonin receptors were revealed. The partial and complete sequences of various types of melatonin receptors were identified in some vertebrates[stripped hairy-footed hamster(*Phodopus sungorus*) and sheep(*Ovis aries*)(Reppert et al., 1994), house mouse(*Mus musculus*)(Roca et al., 1996), Norway rat (*Rattus norvegicus*), human(*Homo sapiens*)(Reppert et al., 1995 a), chicken(*Gallus gallus*) and zebra fish(*Danio rerio*)(Reppert et al., 1995b]. The receptors are members of G protein-coupled receptor superfamily with high percentage of amino acid homology with each other. However, little has been documented about the melatonin receptor in golden hamsters that studied intensively on the efficacy of melatonin in the control of reproduction.

The purpose of the present study was to identify a partial sequence of melatonin receptor in golden hamster and to compare it and the amino acid sequence deduced from it to those reported in other animals. The specificity of the expression of melatonin receptor was examined in various tissues. The reproductive parameters depending upon photoperiods were also investigated.

MATERIALS AND METHODS

1. Animals and Tissue Preparation

Adult male golden hamsters(*Mesocricetus auratus*) were kept in a controlled environment consisting of 14 hours of light and 10 hours of dark(14L:10D, lights on at 0600 h) and a temperature of 22 ± 1℃. They were provided with food and water *ad libitum*. Their sexual activities were maintained in the long photoperiod before using. When they were moved to appropriate photoperiods, they were placed in the cages within the light -protection breeding box in which the lights were controlled by timers and ventilations were always available.

Following the decapitation, various organs(see results) including hypothalamus containing suprachiasmatic nucleus and pituitary gland containing pars tuberalis, were isolated and subjected to the RT-PCR mentioned below. Half of male golden hamsters(8 week old) housed in LP(14L:10D) were transferred to SP(10L:14D, lights on at 0800 h) and the others were remained in the same photoperiod. After 10 weeks, animals from LP or SP were sacrificed by decapitation and trunk blood was taken. At the same time testes were removed, weighed, and subjected to routine histological examination. Blood was collected by cardiac puncture or decapitation, where appropriate. It was allowed to clot in the refrigerator overnight. After centrifugation, serum was stored frozen(−20℃) until assay for hormones.

2. Total Messenger RNA(mRNA) Extraction

Total cytoplasmic mRNA was extracted with acid guanidinium thiocyanate-phenol- chloroform(AGPC) method(Chomczynski & Sacchi, 1987). Briefly, each tissue of the hamster was homogenized in 600 μl of denaturing solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate(pH 7), 0.5% N-lauroyl sarcosine, and 0.1 M 2-mercaptoethanol. Sixty μl of 2 M sodium acetate(pH 4), 600 μl of water-saturated phenol, and 120 μl of chloroform-isoamyl alcohol mixture(49:1) were added. After cooling on ice for 15 min, the samples were centrifuged at 10,000 g at 4℃ for 20 min and precipitated with ethanol. Following washing with 70% ethanol, the RNA pellet was dried under vacuum and dissolved in 20 μl of sterilized distilled water. RNA content was then quantified at A_{260} absorbency. The optical density(OD) ratio of A_{260} to A_{280} ranged from 1.8 to 2.0.

3. Reverse Transcription-polymerase Chain Reaction(RT-PCR)

First strand complementary deoxyribonucleic acid(cDNA) was

synthesized from 0.5 μg of total mRNA prepared as above. The mRNA was mixed with transcription buffer using 0.5 μg of random primers and 200 U of M-MLV reverse transcriptase in the presence of 0.8 mM DTT, 25 U of RNase inhibitor, and 1 mM dNTPs. After incubation at room temperature for 10 minute, the reaction tubes were allowed for 60 minute at 37℃ followed by 5 minute at 95℃. PCR was performed on 4 μl aliquots of the 10 μl first strand cDNA reaction using Mel_{1a} specific oligo primers. The primer pairs were designed not to posses an intron between exon 1 and exon 2 that is greater than 13 kilobases as reported in the mouse(Roca et al., 1996). They were expected to synthesize a part of the melatonin receptor that is from the middle part of the second intracellular loop to the most part of the third intracellular loop, whose first and last small portions were mostly conserved in some animals. PCR was performed using 50 pmol of each primer, 2.5 U of Taq polymerase in the presence of 200 μM dNTPs and 1.5 mM $MgCl_2$. The PCR reaction cycles were composed of 94℃ for 3 minute at the first stage, 94℃ for 50 second, 50℃ for 50 second, and 72℃ for 1.5 minute for 40 cycles at the second stage, and 72℃ for 10 minute at the last stage. Control reactions were done with 1 μg of total mRNA after either RNase or DNase treatment. To avoid the contamination of the solutions, control reactions were processed without the addition of mRNA.

The PCR product were electrophoresed through 1.5% agarose gels, eluted, and compared to the expected size. The PCR products were directly sequenced by Korea Basic Science Institute/ Kwangju Branch.

4. Measurement of Hormones

Concentrations of FSH in serum were determined by using kits(AH R004) manufactured by Biocode S.A. Briefly, samples and ^{125}I-mAb anti-rat FSH tracer were added to the tubes coated with mouse anti-rat FSH mAB. The tubes were incubated on a horizontal shaker at room temperature for 90 min. The mixture was decanted and washed twice. The radioactivity was counted for 1 min in a gamma counter. All materials were used as recommended by the provider.

Serum LH was assayed by heterologous double antibody postprecipitation radioimmunoassay using materials for rat LH (AH R002) manufactured by Biocode S.A. Antisera used was rabbit anti-rat LH polyclonal antiserum. Precipitating agents were sheep anti-rabbit immunoglobulin mixed with a cellulose polyethylene glycol solution. All materials were used as recommended by the provider. Samples and anti-rat LH antiserum was added to the tubes. The tubes were mixed with a vortex mixer and incubated overnight at room temperature. ^{125}I-rat LH was added to the tubes, mixed with a vortex mixer, and incubated at 37℃ for 3 hours. Following addition of PEG-second antibody the tubes were mixed and incubated at room temperature for 30 min. All tubes were spun at 1,500 g for 15 min. The supernatant was removed and the tubes were counted for 1 min. Serum levels of melatonin was determined by Green Cross Reference Lab.

5. Data Analysis

The sequence of the melatonin receptor gene was analyzed for the homology. Amino acid sequence was at the same time deduced from the sequence reported from other animals. Homology was determined by the numbers of either nucleotides or amino acids those were identical to the sequences of hamster melatonin receptor.

RESULTS

The RT-PCR products of melatonin receptors were shown in Fig. 1. The RT-PCR products were not eliminated in samples treated with DNase but eliminated by the treatment of RNase prior to RT-PCR(data not shown). Contamination was not occurred because any RT-PCR product was not seen in the absence of mRNA. The size of RT-PCR products was determined by the molecular marker(lane 1, 100 base pairs). The size of RT-PCR products of melatonin receptors were a little above the 300 base pair of the marker as expected. The outcome of DNA sequenc-

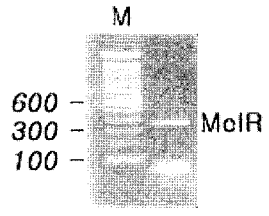

Fig. 1. Reverse transcription polymerase chain reaction(RT-PCR) products of melatonin receptor in golden hamster(*Mesocricetus auratus*). M, 100 base pair marker; MelR, melatonin receptor.

```
Nucleotide sequence  c aag tac gac cga ctc tac agt aac aag aat          31
Amino acid sequence    K  Y  D  R  L  Y  S  N  K  N                   10

tcc ctc tgc tac gtg ttc ctg ata tgg gtg ttg acg cta gtc gcc            76
 S  L  C  Y  V  F  L  I  W  V  L  T  L  V  A                          25

atc atg ccc aac ctg caa acc gga act ctc cag tat gat ccc cgg           121
 I  M  P  N  L  Q  T  G  T  L  Q  Y  D  P  R                          40

atc tac tcc tgt acc ttc aca cag tct gtc agc tcg gtg tac acg           166
 I  Y  S  C  T  F  T  Q  S  V  S  S  A  Y  T                          55

ata gca gtg gtg gtt ttc cat ttc atc gtg cct atg att atc gtc           211
 I  A  V  V  V  F  H  F  I  V  P  M  I  I  V                          70

atc ttc tgc tac tta aga atc tgg atc ctg gtt ctt cag gtc cga           256
 I  F  C  Y  L  R  I  W  I  L  V  L  Q  V  R                          85

cgg agg gtg aaa ccc gac agc aaa ccc aga ctg aag cca cag gac           301
 R  R  V  K  P  D  S  K  P  R  L  K  P  Q  D                         100

ttc agg aa          309
 F  R               102
```

Fig. 2. A partial sequence of melatonin receptor identified by reverse transcription polymerase chain reaction in golden hamster (*Mesocricetus auratus*). Amino acids are symbolized by a single letter.

ing of RT-PCR products of melatonin receptors turned out 309 nucleotides as expected, as shown in Fig. 2. The amino acids are deduced to be 102 in which the first identified nucleotide and the last two nucleotides were not translated.

Fig. 3 demonstrates a comparison of golden hamster melatonin receptor gene to the melatonin receptor genes reported in some mammals. The nucleotide sequence analysis of melatonin receptor of golden hamster shows a homology of 96%, 92%, 88%, 83%, and 80% with stripped hairy-footed hamster, house mouse, Norway rat, human, and sheep, respectively.

As shown in Fig. 4, the amino acid sequence deduced displays a homology of 98%, 91%, 91%, 84%, and 81% with the same order of animals mentioned above.

Table 1 demonstrates tissue specific expression of melatonin receptor gene in golden hamster. Melatonin receptors were strongly detected in blood sample, moderately in hypothalamus, pituitary containing pars tuberalis, and spleen, and weakly in cerebellum, liver adrenal, and testes. They were not detected in brain cortex, muscle, heart, and pancreas.

Fig. 5 shows the profound impact of photoperiods on the paired testicular weights. The animals housed in LP had large testes whereas the animals exposed to SP had small testes. Representative histological examination showed that spermatogenesis was fully developed in the large testes but it was undeveloped in small testes(Fig. 6).

The changes of the pineal hormone melatonin were shown in Fig. 7. As expected, the levels of melatonin in blood was pretty

Table 1. Tissue specificity of melatonin receptor detected by reverse transcription polymerase chain reaction(RT-PCR) in golden hamster. The more numbers of + symbols indicate higher degree of expression of melatonin receptor. +/− indicates a doubtful detection and - expresses no detection. Hypothalamus and pituitary were excised to contain suprachiasmatic nucleus and pars tuberalis, respectively

Tissues	Degree of expression
Hypothalamus	+++
Cortex	−
Pituitary	+++
Cerebellum	+/−
Blood	+++++
Spleen	++
Liver	+/−
Muscle	−
Heart	−
Adrenal	+
Kidney	−
Pancreas	−
Testes	+

```
M. auratus      caagtacgaccgactctacagtaacaagaattccctctgctacgt  45
P. sungorus     ··········a····g····························
M. musculus     ··········aa·a·a·············c··g··········
R. norvegicus   ·····t··ta·ga·a·····················g·····
H. sapiens      ··········aa···g·····c·g·····c·············
O. aries        ··ga····g·aag··g··t··cgg··c·················

M. auratus      gttcctgatatgggtgttgacgctagtcgccatcatgcccaacct  90
P. sungorus     ·····················t·············t···t·
M. musculus     ·············a··c····a··ca·················
R. norvegicus   ·············acac····a··ca·a···············
H. sapiens      ·c····c·····c·cc·····g·cg···g··c··········
O. aries        ·········c···ac·c·····c··g··g···g··········

M. auratus      gcaaaccggaactctccagtatgatccccggatctactcctgtac  135
P. sungorus     ·····t·····································
M. musculus     ·············a·····c·····················
R. norvegicus   ····················c··c·················
H. sapiens      c·gtg·a··g··········c··c··ga·········g··c··
O. aries        ·tgtgtg··g··c··g·····c··c··ga·····t·····

M. auratus      cttcacacagtctgtcagctcggtgtacacgatagcagtggtggt  180
P. sungorus     ·····g··a···········c···········c·····
M. musculus     ·····c·············a·c·····················
R. norvegicus   ·····c·····c·········c·········t··cc·····
H. sapiens      ····g·c·····c·····c·cc·····c··c··c·····
O. aries        ·····g·····c·····a·cc·····c··c·····

M. auratus      tttccatttcatcgtgcctatgattatcgtcatcttctgctactt  225
P. sungorus     ·····································
M. musculus     ···························t·····
R. norvegicus   ··········g·a··c··a·····t····ct·····
H. sapiens      ·····c···c····c··c·····c··a··········t···c·
O. aries        g···········a··t··g···c·cg·a···g·····t···c·

M. auratus      aagaatctggatcctggttcttcaggtccgacggagggtgaaacc  270
P. sungorus     ·····································
M. musculus     ···g··a···g·····c·····a·····
R. norvegicus   ···g··a·····················a·····a·····
H. sapiens      g·····a·····c·····a···a·····
O. aries        g·····gc·····a··t···a·····

M. auratus      cgacagcaaacccagactgaagccacaggacttcaggaa  309
P. sungorus     ·····g·····a·····
M. musculus     ·····a···g····a·····c·····
R. norvegicus   g·····a·····g·····
H. sapiens      t···c·····a·····a·····
O. aries        g····a·····g·a·····c·····
```

Fig. 3. A comparison of nucleotides of melatonin receptor reported in a variety of animals. *M. auratus* : *Mesocricetus auratus*(Golden hamster), *P. sungorus* : *Phodopus sungorus*(Striped hairy-footed hamster): 96%, *M. musculus* : *Mus musculus*(house mouse): 92%, *R. norvegicus* : *Rattus norvegicus*(Norway rat):88%, *H. sapiens* : *Homo sapiens*(human): 83%, *O. aries* : *Ovis aries*(sheep): 80%.

low in the presence of light and high in the darkness. The duration of elevated melatonin was proportional to the duration of darkness. The animals exposed to SP showed longer period of elevated melatonin compared to animals housed in LP. Figs. 8 and 9 showed the serum levels of FSH and LH. The animals moved to SP displayed dramatically reduced level of FSH and

```
M. auratus       KYDRLYSNKNSLCYVFLIWVLTLVAIMPNLQTGTLQYDPRIYSCT  45
P. sungorus      ·····························································
M. musculus      ···KI·····················M···I·····················
R. norvegicus    ····I·····················T···I·····················
H. sapiens       ···K···S·····L···L···A·VL···RA·····
O. aries         R·GK···GT·····T·····V···CV·····

M. auratus       FTQSVSSVYTIVVVVFHFIVPMIIVIFCYLRIWILVLQVRRRVKP  90
P. sungorus      ·····A···A·····
M. musculus      ·····A···A·····V·····
R. norvegicus    ·····A···AL·····V·····T·····
H. sapiens       ·A·····A···A·····L·····Q····
O. aries         ·····A···A·····LV·V·····A·····WK···

M. auratus       DSKPRLKPQDFR  102
P. sungorus      ···········
M. musculus      ·N··K·····
R. norvegicus    ····K·····
H. sapiens       ·R··K·····
O. aries         ·N··K·
```

Fig. 4. A comparison of amino acids of melatonin receptor reported in a number of animals. *M. auratus*: *Mesocricetus auratus*(golden hamster), *P. sungorus: Phodopus sungorus*(striped hairy-footed hamster): 98%, *R. norvegicus: Rattus norvegicus*(Norway rat): 91%, *M. musculus: Mus musculus*(house mouse): 91%, *H. sapiens: Homo sapiens*(human): 84%, *O. aries: Ovis aries*(Sheep): 81%.

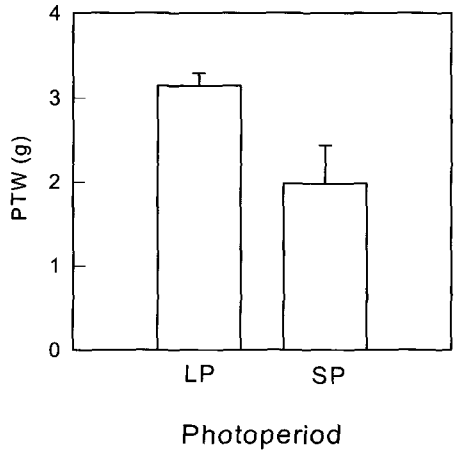

Fig. 5. Paired testicular weights(PTW) of golden hamsters exposed to each photoperiods. LP, long photoperiod; SP, short photoperiod.

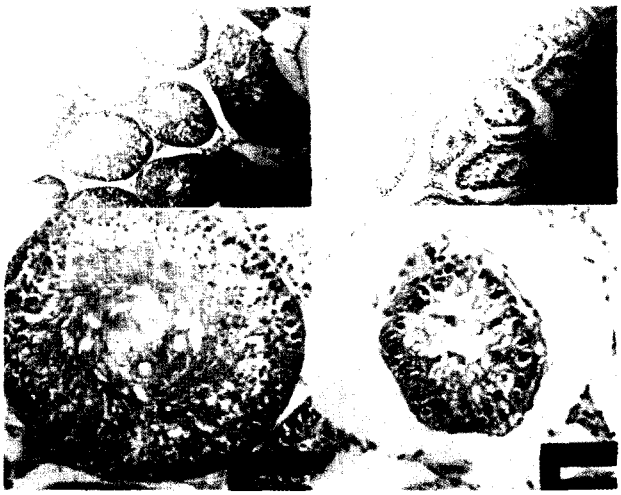

Fig. 6. Histological examination of testes of golden hamsters exposed to each photoperiod. Left, reproductively active testis of golden hamsters housed in LP(14L:10D). Right, Reproductively inactive testis of golden hamsters moved to SP(10L:14D). Scale bars = 50 μm.

LH. The regression of reproductive activities is apparently related to the serum levels of melatonin and reproductive hormones. The expression of melatonin receptor was not affected by the photoperiods.

DISCUSSION

The melatonin receptor gene in golden hamster was identified and compared to those reported in other animals. Melatonin receptors were obviously detected in hypothalamus, pituitary containing pars tuberalis, blood, and spleen. Although the testicular weights and the levels of reproductive hormones were dramatically affected by photoperiods, the expression of melatonin receptor was not markedly changed by them.

The comparison of nucleotides and the amino acids deduced

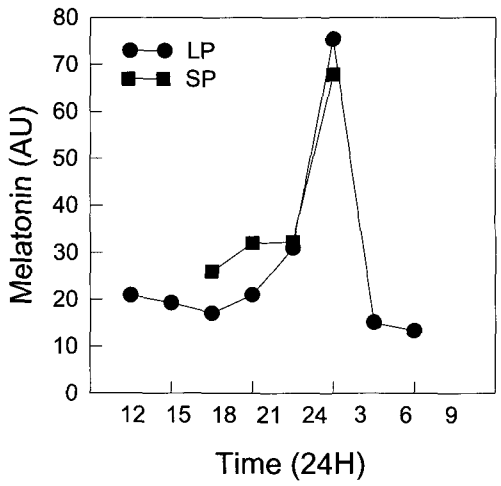

Fig. 7. Serum melatonin levels. Golden hamsters housed in LP (14L:10D) were bled every 3 hour during 1 full day and those in SP(10L:14D) during only dark period. Melatonin concentrations are expressed by arbitrary unit(AU).

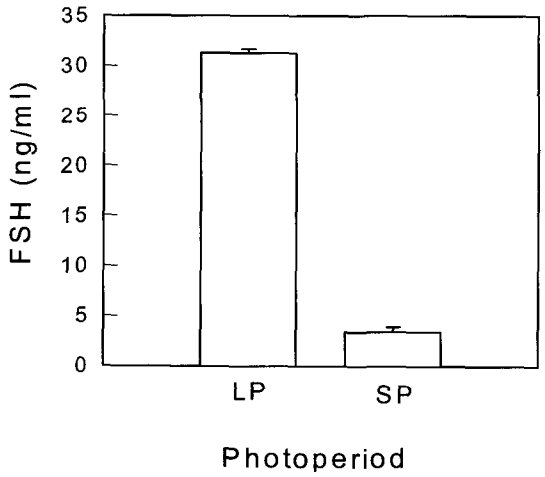

Fig. 8. Serum follicle-stimulating hormone levels. LP, long photoperiod; SP, short photoperiod.

provide us with an evolutionary aspect of the melatonin receptor gene. Tissue specific expression of melatonin receptor provides a novel molecular insight to examine intracellular signaling pathways about the numerous potency of melatonin. Furthermore, the identification of melatonin receptor is expected to unveil the plausible relationship between melatonin and GnRH neuron in the regulation of reproduction.

It has long been documented that the pineal hormone melatonin is a unique mediator to transmit the photoperiodic information into the animals(Reiter, 1991b). In all mammals, the

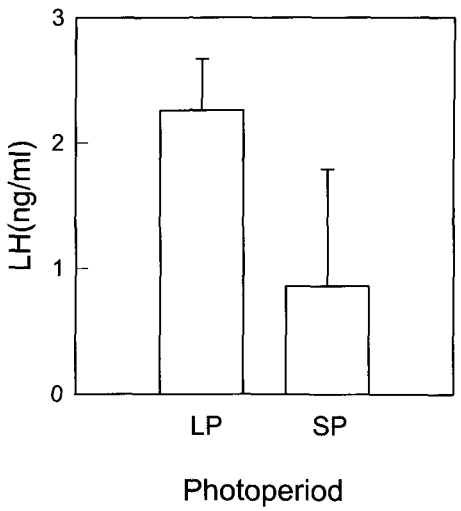

Fig. 9. Serum luteinizing hormone levels. LP, long photoperiod; SP, short photoperiod.

duration of elevated level of melatonin is proportional to the length of dark period(Stetson & Watson-Whitmyre, 1986). Melatonin is also known to be released immediately after synthesis due to hydrophobic nature of the molecule. When the animals are moved from LP to SP, the pineal melatonin content expands gradually. Even in constant darkness, the animals show a similar pattern of melatonin production observed in the animals in light -dark cycle. The exposure of animals to light at night when melatonin levels are high abruptly curtails pineal melatonin production and causes a rapid decline in tissues and blood levels of the hormone. Therefore, synthesis of melatonin is controlled by the light that suppresses it. But even in unusual situation such as constant darkness, the melatonin is not produced continuously during the entire dark period but synthesized in a limited period like the dark period of the photoperiod that animals has previously been experienced. These observations imply that melatonin not only affects the sexual activity but also may act on other putative functions.

Apart from the action of melatonin as a mediator of photoperiod in regulating seasonal reproduction, it has led to the realization of many other of its biological activities(Pierpaoli et al., 1996). It acts as a biological clock to time circadian rhythmicity and a neuromodulator in the retina. In addition, there are a great deal of increasing evidence that the pineal gland through melatonin is potentially involved in the anti-aging and life-prolonging effects via the anti-oxidative mechanism. Melatonin

appears to exert an inhibitory effects on the growth of tumor and cancer cells and participates in the control of the cardiovascular function as an endogenous hypotensive/bradycardiac factor in the central nervous system. It plays an important supportive role of the immune system. Virtually, melatonin has multifunctional roles at various levels from molecule to whole body.

The potency of melatonin is exerted through its receptor that was recently identified in some animals. Melatonin receptor has been in common displayed in the hypophyseal pars tuberalis and the hypothalamic suprachiasmatic nucleus by ^{125}iodomelatonin binding and RT-PCR methods from most animals studied (Ebisawa et al., 1994; Reppert et al., 1994; Roca et al., 1996; Reppert et al., 1995a; 1995b; Morgan et al., 1994). These sites are thought to be important for reproductive and circadian responses to melatonin, respectively. The suprachiasmatic nucleus is the site of a master circadian clock in mammals and the pars tuberalis is a thin strip of pituitary tissue on the surface of the median eminence and anterior pituitary and is the only site containing detectable levels of ^{125}iodomelatonin binding in all seasonal breeding species examined to date. Most of the work identifying melatonin effects on reproduction has been accomplished using hamsters(*Mesocricetus* and *Phodopus*), but methods for genetic manipulation of hamsters do not exist. Therefore, even the partial discovery of melatonin receptor gene in the golden hamster is exceptionally precious.

It has been known that melatonin receptor is coupled to G-protein, particularly G_i protein. The binding of melatonin to the melatonin receptor inhibits forskolin-evoked increase of cyclic AMP levels in the cells(Ebisawa et al., 1994; Reppert et al., 1994; Roca et al., 1996; Reppert et al., 1995a; Liu et al., 1997). Concurrently, it has been reported that melatonin potentiates the activation of phospholipase C to affect the intracellular Ca^{++} levels(Godson & Reppert, 1997). Thus the identification of melatonin receptor in the golden hamster facilitates to define the intracellular signaling pathways activated by melatonin.

The partial nucleotide sequence of golden hamster melatonin receptor gene shows a homology of 96%, 92%, 88%, 83%, and 80% with stripped hairy-footed hamster(*Phodopus sungorus*) (Reppert et al., 1994), house mouse(*Mus musculus*)(Roca et al., 1996), Norway rat(*Rattus norvegicus*), and human(*Homo sapiens*) and sheep(*Ovis aris*)(Reppert et al., 1995a), respectively. The results clearly indicates high percentage of similarity in the intraspecies, between golden hamster and stripped hairy-footed hamster. Low similarities are demonstrated in comparison to the human and sheep.

The amino acid sequence deduced displays a homology of 98 %, 91%, 91%, and 84% with the same order of animals mentioned above. There are similar trends of both the nucleotide and the amino acid sequence of melatonin receptors within the range of examination. These findings may suggest an evolutionary aspect of the melatonin receptor gene.

Melatonin receptors were strongly detected in the samples of blood, moderately in hypothalamus, pituitary gland, and spleen, and weakly in cerebellum, liver, adrenal gland, and testes. They were not detected in brain cortex, muscle, heart, and pancreas. The results are similar to the results reported previously by ^{125}iodomelatonin binding and RT-PCR methods from most animals studied(Ebisawa et al., 1994; Reppert et al., 1994; Roca et al., 1996; Reppert et al., 1995a; 1995b; Morgan et al., 1994). The findings will open new wide window to investigate the numerous putative effects of melatonin at the level of molecules(Pierpaoli et al., 1996). The lack of profound daily change in the expression of melatonin receptor observed in golden hamsters is consistent with the previous report(Recio et al., 1998). whether melatonin regulates melatonin receptor gene transcription is still under investigation(Guerrero et al., 2000; Heideman et al., 2001).

A portion of the melatonin receptor gene was also reported in the GT1-1 cells, which release GnRH in a pulsatile fashion that is an intrinsic property of GT1-1 GnRH neuronal cell lines(Weiner & Escalera, 1993). The melatonin receptor gene detected in GT1-1 cells is completely identical to the reported mouse melatonin receptor(Roca et al., 1996). Thus the presence of melatonin receptor in GT1-1 cell suggests possible function to regulate the synthesis or release of GnRH.

In summary the melatonin receptor gene in golden hamster and GT1-1 cells was presented.

The comparison of nucleotides and the amino acids deduced provide us with an evolutionary aspect of the melatonin receptor gene. Tissue specific expression of melatonin receptor provides a novel molecular insight to examine intracellular signaling pathways about the numerous potency of melatonin. Furthermore, the identification of melatonin receptor in GT1-1 cells is expected to unveil the plausible relationship between melatonin

and GnRH neuron in the regulation of reproduction. The expression of melatonin receptor was not changed drastically by photoperiod. The results suggest that the action of melatonin in regulating reproduction might be mainly due to the affinity of melatonin receptor rather than the density of melatonin receptor.

ACKNOWLEDGEMENTS

The authors thank Prof. Yong-Dal Yoon and his colleagues for their help in analyzing reproductive hormones FSH and LH. We wish to thank Green Cross Reference Lab for determining melatonin in serum. We also would like to appreciate Korea Basic Science Institute/Kwangju Branch for determining the nucleotide sequence of melatonin receptor.

REFERENCES

Choi D (1996) Reproductive physiology of pineal hormone melatonin. Korean J Zool 39:337-351.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.

Ebisawa T, Karne S, Kerner MR, Reppert SM (1994) Expression cloning of a high-affinity melatonin receptor from Xenopus dermal melanophores. Proc Natl Acad Sci USA 91:6133-6137.

Elliott JA (1976) Circadian rhythms and photoperiodic time measurement in mammals. Fed Proc 35:2339-2346.

Gaston S, Menaker M (1967) Photoperiodic control of hamster testis. Science 158:925-928.

Godson C, Reppert SM (1997) The melatonin receptor is coupled to parallel signal transduction pathway. Endocrinology 138: 397-404.

Guerrero HY, Gauer F, Schuster C, Pevet P, Masson-Pevet M (2000) Melatonin regulates the mRNA expression of the mt(1) melatonin receptor in the rat pars tuberalis. Neuroendocr 71:163-169.

Heideman PD, Bierl CK, Sylvester CJ (2001) Photoresponsive Fischer 344 rats are reproductively inhibited by melatonin and differ in 2-[^{125}I] iodomelatonin binding from nonresponsive Sprague-Dawley rats. J Neuroencr 13:223-232.

Jackson FL, Heindel JJ, Preslock JP, Berkowitz AS (1984) Alterations in hypothalamic content of luteinizing hormone -releasing hormone associated with pineal-mediated testicular regression in the golden hamster. Biol Reprod 31:436-445.

Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM (1997) Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. Neuron 19:91-102.

Maywood ES, Lindsay JO, Karp J, Powers JB, Williams LM, Titchener L, Ebling J, Herbert FJP, Hastings MH (1991) Occlusion of the melatonin-free interval blocks the short day gonadal response of the male syrian hamster to programmed melatonin infusions of necessary duration and amplitude. J Neuroendocr 3:331-337.

Morgan PJ, Barrett P, Howell HE, Helliwell R (1994) Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Intl 24:101-146.

Pierpaoli W, Regelson W, Colman C (1996) The Melatonin Miracle; Nature's age-reversing, disease-fighting, sex-enhancing hormone. G.K. Hall & Co. Thorndike Maine pp 1-438.

Recio J, Gauer F, Schuster C, Pevet P, Masson-Pevet M (1998) Daily and photoperiodic 2-^{125}I-melatonin binding changes in the pars tuberalis of the Syrian hamster(*Mesocricetus auratus*): effect of constant light exposure and pinealectomy. J Pineal Res 24:162-167.

Reiter RJ (1991a) Pineal gland, Interface between the photoperiodic environment and the endocrine system. Trends Endocrinol Metab 2:13-29.

Reiter RJ (1991b) Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocrine Rev 12:151-180.

Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF (1995a) Molecular characterization of a novel melatonin receptor expressed in human retina and brain: the mel_{1b}-melatonin receptor. Proc Natl Acad Sci USA 92:8734 -8738.

Reppert SM, Weaver DR, Cassone VM, Godson C, Kolakowski Jr LF (1995b) Melatonin receptors are for birds: molecular analysis of two receptor subtypes differentially expressed in chicken brain. Neuron 15:1005-1015.

Reppert SM, Weaver DR, Ebisawa T (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian response. Neuron 13:1177-1185.

Roca AL, Godson C, Weaver DR, Reppert SM (1996) Structure, characterization, and expression of the gene encoding the mouse Mel_{1a} melatonin receptor. Endocrinology 137:3469-347.

Steger RW, Matt K, Bartke A (1985) Neuroendocrine regulation of seasonal reproductive activity in the male golden hamster. Neurosci Biobehav Rev 9:191-201.

Stetson MH, Tay DE (1983) Time course of sensitivity of golden hamsters to melatonin injections throughout the day. Biol Reprod 29:432-438.

Stetson MH, Watson-Whitmyre M (1984) Physiology of the pineal and its hormone melatonin in annual reproduction in rodents. In: Reiter, RJ(ed.), The pineal Gland. Raven press, New York, pp 109-153.

Stetson MH, Watson-Whitmyre M (1986) Effects of exogenous and endogenous melatonin on gonadal function in hamsters. J Neural Transm 21(Suppl) 55-80.

Weiner RI, Escalera GM (1993) Pulsatile release of gonadotropin releasing hormone(GnRH) is an intrinsic property of GT1 GnRH neuronal cell lines. Human Reproduction 8:13-17.