Proc 4th Ann Conf Kor Soc Dev Biol, July 3, 1998, pp 6-7.

Regulation of Placental Lactogen mRNA Levels by Melatonin in the Rat Placenta

Chae Kwan Lee, Han Seung Kang, Chang Sook Shin, Bu Il June, Sung Goo Kang
Department of Biology, Inje University, Kimhae, Korea.

INTRODUCTION

Melatonin is a hormone produced rhythmically by the pineal gland (Arendt, 1995). The melatonin rhythm is coordinated to the external light-dark cycle, with high levels produced only at night. In mammals, the pineal melatonin rhythm is driven by an endogenous circadian clock that resides in the suprachiasmatic nuclei (SCN) of the hypothalamus (Klein et al, 1991). The SCN receve sensory input from the retina that keeps the circadian clock and resultant melatonin rhythm entrained to the 24-h day.

Melatonin has two well described biological effects (Arendt, 1995, Reppert and Weaver, 1995). First, melatonin controls the annual timing of reproductive function in seasonally breeding mammals. This is accomplished through the nocturnal increase in the hormone, which provides a measure of the duration of the night. Melatonin also speeds adjustment of the circadian clock to changes in the light-dark cycle. Melatonin elicits its biological effects through high affinity G protein-coupled receptors (Reppert and Weaver, 1995). A significant advance in this area has been the recent cloning of the complementary DNAs (cDNAs) for a family of G protein-coupled melatonin receptors in vertebrates (Reppert et al, 1994). Cloning studies in mammals have identified two high affinity melatonin receptor subtypes, the Mel_{1a} and Mel_{1b}. Mel_{1a} receptor is expressed in SCN and hypophyseal pars tuberalis (PT), presumed sites of the circadian and some of the reproductive effects of melatonin, respectively.

Mel_{1b} receptor massenger RNA is not detected in brain by in situ hybridization, but has been identified in human retina and brain by RT-PCR. A third receptor, Mel_{1c} has been cloned from zebrafish, Xenopus and chickens, but not from mammals. In mammlians, the reproductive effect and circadian rhythm of melatonin mediated by Mel_{1a} receptor in the PT and SCN. Throughout these process, Mel_{1a} mediated the changes of prolactin gene expression (Lincoln and Clarke, 1994). The expression of Mel_{1a} was reported in the rat lives and other tissue (Menendez-Palaez et al, 1993, Acuna-Costroviejo et al, 1995). In this study, we were interested in the local expression, gestational profile of Mel_{1a} receptor and circadian rhythm of placenral lactogen Iv (PL-Iv), II (PL-II), placental prolactin like protein C (PLP-C) and Mel_{1a} receptor genes in the rat placental (*in vivo*). Also we investigate the effect of melatonin (*in vitro*) on expression PL-Iv, PL-II, PLP-C genes using the northern blot analysis.

MATERIALS & METHODS

Animals and tissue preparation. Pregnancy of Sprague Dawley rats were checked with the presence of a copulatory plug or sperms in the vaginal smear and defined as pregnant day 0. Rats were sacreficed at pregnant day 12 and 20 to examined the gestational profile of Mel_{1a} receptor gene. Also, rats were sacreficed at PM4:00, PM10:00, AM4:00 and AM10:00 during the gestational day 19 and 20 to examined the circadian rhythm of Mel_{1a}, PL-Iv, PL-II, PLP-C genes and a part of divisions were prepared for tissue culture.

Placental tissue culture. Placentas removed from the sacreficed pregnant rats were devided to the junctional zone and labyrinth zone and minced (1mm in size) using the micro forceps. Minced tissues were treated with lysis buffer to avoide the contamination of blood compononts and cultured in MEM for 2 hours treated with melatonin agonist (chloromelatonin).

Northern blot analysis Total RNA was extracted according to the guanidium phenol chloroform method. Hybridization was carried out for 12 hours at 55℃ with ^{32}P labeled Mel_{1a} or PL-Iv or PL-II or PLP-C cDNA probes after 2 hour-prehybridization. Probes were synthesied using the random primer labelling kit (Pharmacia).

RESULTS & DISCUSSION

Northern blot analysis showed that Mel_{1a} receptor gene expressed (mainly in the labyrinth zone) in the rat placenta. mRNA level was higher at pregnant day 12 than at pregnant day 20 (data not shown). Circadian rhythm of Mel_{1a} mRNA level was higher at nighttime (AM4:00) than at daytime (PM4:00) in the labyrinth zone. In the junctional zone, however, Mel_{1a} mRNA level was higher at daytime (PM4:00) than at nighttime (AM4:00) (Fig.1). Circadian rhythn of Mel_{1a} in the placental labyrinth zone well coincident with the change of blood melatonin concentration during daytime and nighttime. But in the placental junctional zone, circadian rhythm of Mel_{1a} has an reciprocal proportion to the change of blood melatonin concentration during daytime and nighttime.

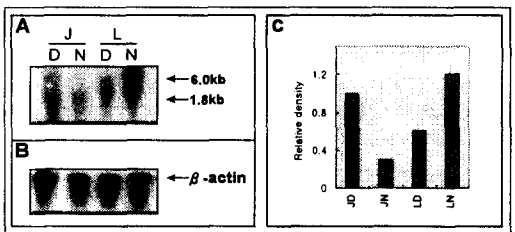

Fig.2. Changes of melatonin receptor (Mel_{1a}) mRNA level at day (D), (PM 4:00) and night (N), (AM 4:00) in the rat placental junctional (J) and labyrinth (L) zone. (A) Northern blot analysis of Mel_{1a}. Total RNA (30ug) was electrophoresed on an formaldehyde gel. Tmasfered to nylon paper and hybridized with ^{32}P labeled Mel1a cDNA probe. β-actin was hybridized to certify the aberration of analysed total RNA. (C) Northern signals were quantified by scanningdensitometry and expressed as relative units over J-D value of 1.0.

PL-Iv gene mainly expressed in the junctional zone and mRNA level was higher at daytime than at nighttime in the junctional zone and labyrinth zone. Also, PLP-C mainly expressed in the junctional zone. However, significant change was not detected at daytime and nighttime. PL-II gene mainly expressed in the labyrinth zone and mRNA level was higher at daytime than at nighttime in the labyrinth zone. In the junctional zone, However, PL-II mRNA level was higher at nighttime (Fig.2). Circadian rhythm of PL-II gene has an reciprocal proportion to the circadian rhythm of Mel_{1a} receptor gene in the placental junctional zone and labyrinth zone.

In the cultured placenta, melatonin agonist (chloromelatonin) decreased PL-Iv, PL-II, PLP-C mRNA levels in dose dependently.

These data suggested that Mel_{1a} is one of the main factors that control the circadian rhythms of PL-Iv, PL-II, PLP-C genes in the rat placenta response to the repeted changes of blood melatonin concentration during the late pregnancy.

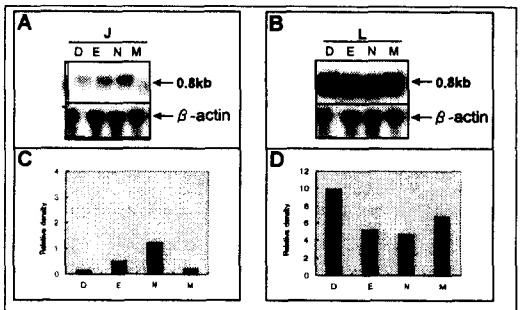

Fig.2. Changes of PL-II mRNA level at day and night in the rat placenta. Pregnant rat was sacrificed at PM 4:00 (D), PM 10:00 (E), AM 4:00 (N), AM 10:00 (M) and purified total RNA from placental junctional zone (J) and labyrinth zone (L). Northern blot analysis of placental junctional zone (A) and labyrinth zone (B). Total RNA was electrophoresed on an formaldehyde gel. Tmasfered to nylon paper and hybridized with ^{32}P labeled PL-II cDNA probe. β-actin cDNA probe was hybridized to certify the aberration of total RNA. Northern signals were quantified by thr ratio of PL-II/ β-actin and expressed as relative units over JD value of 1.0.

REFERENCES

Acuna-Costroviejo D, Reiter RJ, Menendez-Palaez A, Pablos MI, and Burgos A (1995) *J Pineal Res* 16: 100-112.

Arendt J (1995) Melatonin and the Mammlian Pineal Gland. Chapman and Hill, London.

Klein DC, Moore RY, and Reppert SM (1991) Suprechiasmatic Nucleus: The Mind's Clock. Oxford Press, New York.

Lincoln GA and Clarke IJ (1994) *J Endocrinol* 6: 251-260.

Menendez-Palaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, and Tan DX (1993) *J Cell Biochem* 54: 1-10.

Reppert SM and Weaver DR (1995) *Cell* 83: 1059-1062.

Reppert SM, Weaver DR, and Ebisawa T (1994) *Neuron* 13: 1177-1185.