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Pineal Gland (Melatonin) Affects the Parturition Time, but not Luteal Function and Fetal Growth, in Pregnant Rats

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Abstract. The purpose of the present study was to investigate the role of pineal gland (melatonin) on parturition time, luteal function, and fetal growth in pregnant rats. Cycling rats were subjected to pinealectomy or sham operation under ether anesthesia; and pinealectomized rats immediately underwent implantation of a melatonin capsule (PINX + Mel group) or a vehicle-containing capsule (PINX group), and sham operated rats also underwent implantation of a vehicle-containing capsule (control group). All rats were maintained under the same photoperiod conditions (14 L : 10 D) and were induced pregnancy. Blood samples were obtained on days 7, 12, 15, 17, 19, and 21 of pregnancy to measure serum progesterone concentrations, and parturition times were recorded on days 22 and 23. In the next experiment, pregnant PINX rats received subcutaneous injection of melatonin (10 μg/body) at 08:00 h (PINX + 8 h group) or at 20:00 h (PINX + 20 h group) from day 15 to the end of pregnancy, and parturition times were recorded. Parturition times of rats in the PINX group, the PINX + Mel group or the PINX + 8 h group, but not the PINX + 20 h group, were significantly different compared with those in the control group. Pinealectomy or melatonin implantation did not affect serum progesterone concentrations during pregnancy or the number and weight of fetuses or corpora lutea. The present results indicate that pineal gland (melatonin rhythm) synchronizing with photoperiodic rhythm is likely to be an important determinant of parturition time, but it does not affect progesterone production or fetal growth in pregnant rats.

Key words: Melatonin, Parturition, Photoperiod, Pineal gland, Pregnancy, Rat

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ALTHOUGH the mechanism determining parturition time is unclear, several reports have implicated light (photoperiod) in the control of parturition time in human [1, 2] and rats [3]. Rats give birth during the day-light hours [3], even if the light on/off time is artificially shifted [4, 5]. Continuous darkness abolishes photoperiod timing of parturition [6], and light pulse can delay or advance the time of parturition [7]. Melatonin is synthesized in the pineal gland and is secreted in a photoperiodic manner. It plays an important role in circadian rhythmicity [8] and in reproductive events [9]. Pineal gland (melatonin) is likely to be a photoperiod mediator regulating parturition time in rats [10] and sheep [11]. Viswanathan and Davis [12], however, reported that day- or night-time melatonin injection under the control condition into pregnant hamsters with lesions to the suprachiasmatic nucleus (SCN) does not affect parturition time so that SCN, not melatonin rhythm, determines the time of parturition. Thus, photoperiod is likely to be an important factor to control parturition time,

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but the role of pineal gland (melatonin) in it is still obscure.

Some reports indicate that melatonin affects hormone secretion during pregnancy and fetal survival. Guerra *et al.* [13] reported that pinealectomy induces abortion in pregnant rats. Nir *et al.* [14] reported that pinealectomy affects maternal serum prolactin (PRL) and luteinizing hormone (LH) levels during late pregnancy, but that it does not affect fetal survival. In our previous report, pinealectomy does not affect daily PRL rhythm or concentration in pseudopregnant rats [15]. Thus, the effects of melatonin on maternal endocrinological findings and fetal survival also remain controversial.

In the present study, we examined the role of pineal gland (melatonin) on parturition time, and also luteal function and fetal growth in pregnant rats.

Materials and Methods

Animals and materials

Experimental protocols were reviewed by the Committee for Ethics on Animal Experimentation and were carried out under the Guidelines for Animal Experiments at Yamaguchi University School of Medicine under Law (No. 105) and Notification (No. 6) of the Japanese Government. Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan), 220–290 g, were housed under controlled conditions (lights on from 05:00 h to 19:00 h) with free access to standard rat chow and water, and rats showing at least two consecutive 4-day estrous cycles were used for experiments. Powdered melatonin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Beeswax was purchased from Katayama Chemical Co. (Osaka, Japan), and polyethylene tubing was from Masuda Laboratory Equipment (Osaka, Japan). [^{3}H]-melatonin (37 Mbq/ml) and [^{3}H]-progesterone (37 Mbq/ml) were purchased from Du Pont Co. (Wilmington, DE, USA). Melatonin antiserum (HAC-AA92-03RBP86) was kindly provided by Dr. Katsumi Wakabayashi (Endocrinology Research Center, Gunma University, Japan).

Pinealectomy and melatonin implantation

Pinealectomy was performed according to the method of Hoffman and Reiter [16]. Anesthetised rats were placed in a stereotaxic apparatus, and an incomplete circular cut was made to the dorsal skull with a dental drill. The piece of cranium covering the pineal gland was folded anteriorly, and the pineal gland was removed with fine-tipped forceps. After removal of pineal gland, the bone flap was replaced, and scalp was closed. Melatonin implantation was performed as reported previously [15]. Briefly, melatonin was dissolved in melted beeswax at a concentration of 1 mg melatonin/25 mg beeswax and was loaded into polyethylene tubing (2.50 mm i.d. × 2.75 mm o.d.). After the beeswax had cooled to room temperature, the tubing was cut into pieces 7 mm in length. Vehicle capsules contained only beeswax. The tubing was implanted through a small dorsal-skin incision. Serum melatonin levels were constant up to 3 weeks after implantation and decreased gradually thereafter [15]. Therefore, melatonin or vehicle capsules were replaced every 3 weeks during the duration of experiments.

Experimental design

Cycling rats underwent pinealectomy or sham operation under ether anesthesia between 12:00 h and 16:00 h on diestrus day 1. Pinealectomized rats were divided into two groups and immediately underwent subcutaneous implantation of a melatonin capsule (PINX + Mel group, n = 26) or a capsule containing vehicle (PINX group, n = 31). Rats in the sham group also received a capsule containing vehicle (control group, n = 26). Following the operation, all rats were subjected to the same photoperiod conditions (14 L : 10 D), and estrous cyclicity was examined. Since the effect of melatonin is reported to stabilize 3–5 weeks after melatonin injection [17, 18], rats were housed with males overnight on proestrus of the 8th estrous cycle after the operation. The day a sperm-positive smear was obtained was designated as day 1 of pregnancy.

In the first experiment, blood samples (0.3–0.4 ml) were obtained by subclavicular venipuncture under light ether anesthesia between 12:00 h and 13:00 h on days 7, 12, 15, 17, 19, and 21 of pregnancy. The blood samples were allowed to clot at −4°C, and se-

rum was stored at −20°C until the progesterone assay. On days 22 and 23, parturition time was identified by the birth of the first pup. Some rats in each group were sacrificed under deep ether anesthesia on day 21 of pregnancy, and the number and weights of fetuses or corpora lutea were recorded.

In the second experiment, pregnant PINX rats received subcutaneous injection of melatonin in sesame oil (10 μg/0.2 μl/body) at 08:00 h and oil alone at 20:00 h (PINX + 8 h group, n = 18), or oil alone at 08:00 h and melatonin at 20:00 h (PINX + 20 h group, n = 15) from day 15 to the end of pregnancy. The injected dose of melatonin in oil was decided from the previous reports [19, 20], and we evaluated time-dependent changes of serum melatonin concentrations by the preliminary experiment (Fig. 1). Parturition time in those rats was recorded on days 22 and 23 of pregnancy.

Melatonin and progesterone assay

Serum melatonin concentrations were measured by radioimmunoassay (RIA) as reported previously [15]. Briefly, 2 ml chloroform was added to 0.5 ml serum, and the 1.5-ml organic phase was aspirated, evaporated, and dissolved in 250 μl of assay buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.1% BSA, 0.1% NaN_3). Melatonin antiserum (100 μl, 1 : 30,000) and [^{3}H]-melatonin (5,000 cpm/100 μl) were added to tubes containing standards or samples, and tubes were incubated for 24 h at 4°C. The bound/free separation was performed by the dextran-charcoal method, and the radioactivity of supernatant was measured by a liquid scintillation counter (LSC5100, Aloka). The lower limit of the assay was 2.1 pg /tube, and the intra- and interassay coefficients of variation were less than 10%.

Serum progesterone concentrations were determined by specific RIA as reported previously [21]. The lower limit of the assay was 5.4 pg/tube, and the intra- and interassay coefficients of variation were 7.0% and 14.4%, respectively.

Statistical analysis

Parturition times were analyzed by Fisher's exact probability test. Data of serum progesterone or melatonin concentrations and the number or weight of corpora lutea and fetuses were shown as mean ± SEM, and those data were analyzed by ANOVA and Duncan's new multiple range test.

Results

Serum melatonin concentrations were low during

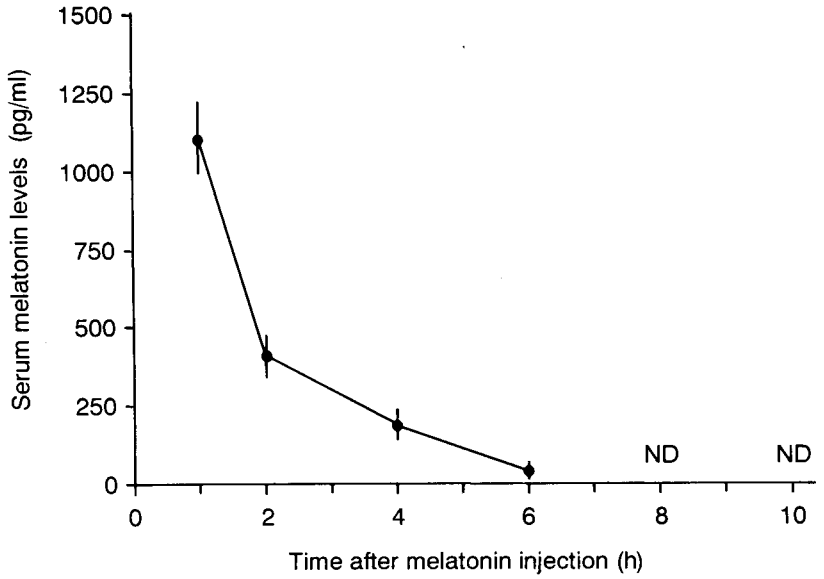


Fig. 1. Time-dependent change of serum melatonin concentrations in pinealectomized rats injected melatonin in sesame oil (10 μg/0.2 μl/body). Vertical lines indicate SEMs of three rats. ND indicates less than lower limit of the assay sensitivity.

the daytime and high at night in the control group (Fig. 2). Concentrations were low during both periods in the PINX group and were high during both periods in the PINX + Mel group (Fig. 2). Pinealectomy or melatonin implantation did not alter estrous cyclicity in any of the rats, and there was no significant difference between the three groups in the ability of rats to conceive.

Parturition times (mean ± 2SD) in control rats were between 12:40 h and 16:38 h on day 22, or 08:10 h and 16:06 h on day 23 of pregnancy (Fig. 3). Seven (36.8%) of 19 rats in the PINX group ($P < 0.01$), 5 (26.3%) of 19 rats in the PINX + Mel group ($P < 0.05$) and 4 (22.2%) of 18 rats in the PINX + 8 h group ($P < 0.05$) initiated parturition at the other times (Fig. 3). However, only 3 (20.0%) of 15 rats in the PINX + 20 h group initiated parturition at the other times; there was no significant difference between the PINX + 20 h group and the control group (Fig. 3).

Changes in serum progesterone concentration during pregnancy did not differ between the three groups (Fig. 4). The number or weight of fetus and corpora lutea was not different between the groups on day 21 (Table 1).

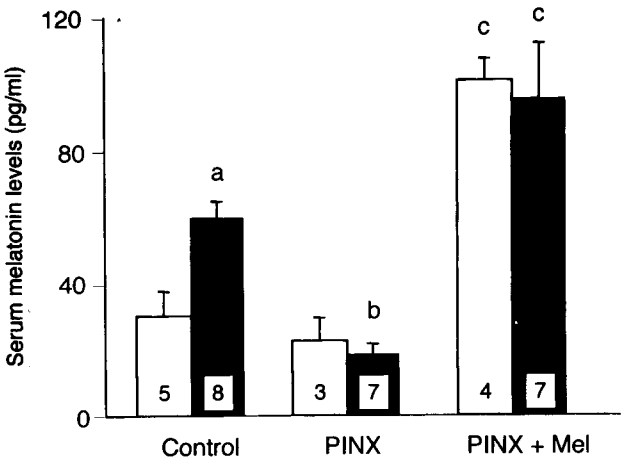


Fig. 2. Serum melatonin levels in each group determined at daytime (12:00 h, open bars) or nighttime (00:00 h, solid bars). Values are mean ± SEM for the number of rats indicated in each bar.
a: $P < 0.05$ vs daytime value. b: $P < 0.05$ vs the nighttime value in control group. c: $P < 0.01$ vs each value in control group.

Discussion

Pregnant rats in the control group showed a daytime preference for parturition in the present study, i.e. afternoon on day 22 or daytime on day 23 of pregnancy. In contrast, rats with constantly low (PINX group) or high serum melatonin levels (PINX + Mel group) showed loss of the daytime preference for parturition. Melatonin injection at 20:00 h to PINX rats, whose melatonin rhythm resembled it in the control rats, could partially recover the daytime preference, and only one of 15 treated rat gave birth at dusk. However, 3 (16.7%) of 18 PINX rats injected melatonin at 08:00 h still gave birth during dark period.

All rats in the present study were housed under the same photoperiodic condition (lights on from 05:00 h to 19:00 h), and more than 60% of rats in any treatment groups still gave birth at day times as in the control group. Bosc [10] reported that melatonin might be a mediator by which the photoperiod regulates parturition time in rats. Although pineal gland may play a role in controlling the time of parturition, photoperiodic stimulation is likely to be a more potent factor for it, and melatonin rhythm synchronizing with photoperiodic rhythm is the most important factor for controlling parturition time. Rats kept in constant darkness from day 7 of pregnancy gave birth at all times on day 22 and 23 of pregnancy [22]. When the light on/off time was altered after conception, pregnant rats tended to give birth during the light period [5, 22]. In those studies, lighting schedules were set on early days of pregnancy. The time shift of parturition was not completed when changing it on day 12 of gestation in pregnant rats [6]. It takes 8–10 days for rats to adjust their rhythm to 8 h photoperiodic time shift [6]. Also, pinealectomy on day 8 of gestation fails to alter the time of parturition in pregnant rats [6]. A critical time period may exist for photoperiodic and/or melatonin rhythm to affect the parturition time. We injected melatonin from day 15 to the end of pregnancy in the present study because serum melatonin concentration increases significantly on day 15 and afterward (unpublished data). However, the day of melatonin injection in the present study might be too late to induce a complete daytime preference for parturition. Moreover, the artificial melatonin rhythm might not be sufficient to induce the daytime preference in PINX rats

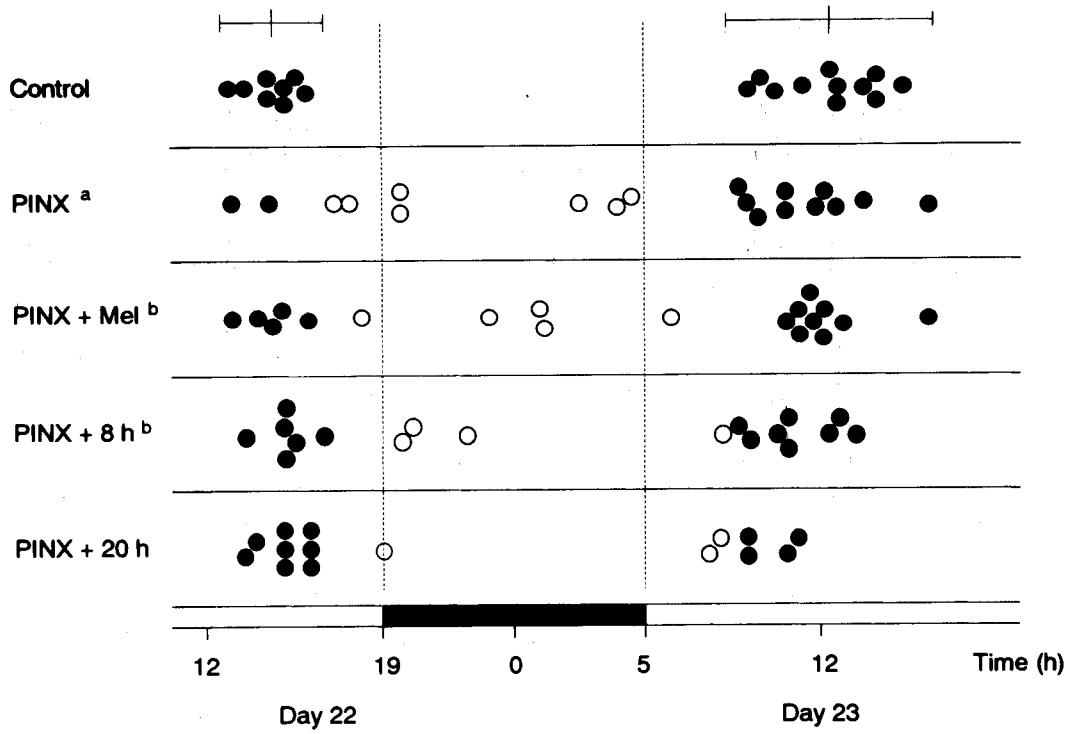


Fig. 3. Effects of pinealectomy or melatonin supplementaion to pinealectomized rats on parturition times. Black dots indicate parturition time of rats who gave birth during mean ± 2SD period of control group, and white dots indicate those of rats who gave birth at the other times. Horizontal lines above control group show mean ± 2SD period of parturition time in control group. Bottom white bar indicates light period, and black bar indicates dark period.
a: $P<0.01$ vs control group. b: $P<0.05$ vs control group.

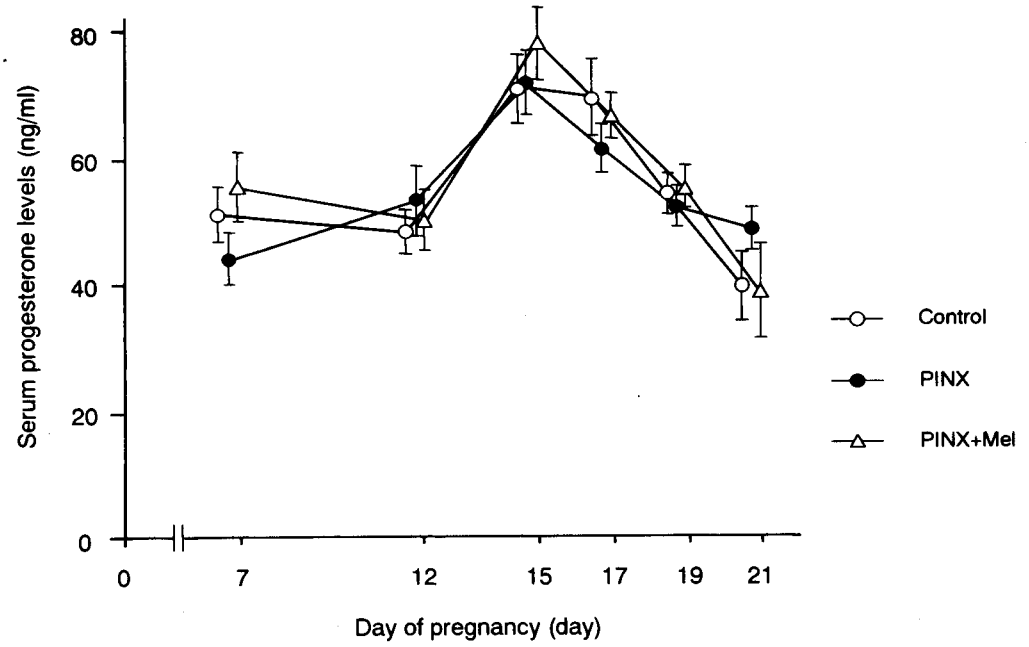


Fig. 4. Changes in serum progesterone levels between days 7 and 21 of pregnancy in the control (open circle), PINX (closed circle) and PINX + Mel (open triangle) groups. Values are means ± SEM for 20 rats in each group.

Table 1. Number and weight of fetuses or corpora lutea in each group on day 21 of pregnancy.

Group	Fetus		Corpora lutea	
	Number	Weight (g)	Number	Weight (mg)
Control (n=6)	12.6±1.5	3.0±0.2	14.8±2.0	4.2±0.6
PINX (n=5)	11.8±0.8	3.0±0.2	16.6±5.3	4.3±0.7
PINX+Mel (n=6)	12.2±2.0	2.9±0.1	14.4±1.1	4.2±1.1

Values are mean ± SEM for 5–6 rats.
PINX: pinealectomy. PINX+Mel: melatonin capsule implantation to pinealectomized rats.

although it resembled the natural rhythm.

The mechanism for melatonin to control parturition time is unclear. The fetus is thought to play an important role in determining parturition time [23]. Melatonin can easily cross the placenta from mother to fetus [24], and the maternal melatonin rhythm informs the fetus of the season and the time of day [25] and affects the neonatal circadian rhythm [20]. Pregnant rats in whom fetuses were subjected to brain aspiration on day 19 no longer exhibit a daytime preference for parturition [26]. Melatonin rhythm synchronizing with photoperiodic rhythm may entrain the fetal rhythm which plays an important role for the control of parturition time.

Neither pinealectomy nor melatonin implantation affected estrous cyclicity, the ability of rats to become pregnant, or fetal growth in our study rats. Serum progesterone levels in the PINX and the PINX+Mel groups, which are dependent on the presence of the maternal pituitary and placental hormones [27–29], were in the same range as those in the control group during pregnancy. Pinealectomy has been reported to increase serum LH levels and to decrease serum PRL levels in pregnant rats during the last few gestational days [14]. Our previous study, however, shows no effects of pinealectomy on daily PRL rhythm or concentration in pseudopregnant rats [15]. Furthermore, rats in the PINX and the PINX+Mel groups ovulated on the day after delivery, as did in the control group (unpublished data). The follicle-stimulating hormone/LH ratio and gonadal steroid production during pregnancy are important for the first ovulation after delivery in rats [30, 31]. Those results indicate that pineal gland (melatonin) may not alter maternal hormone profiles during pregnancy.

In conclusion, pineal gland (melatonin rhythm) synchronizing with photoperiodic rhythm is likely to be an important determinant of parturition time in pregnant rats. Neither pineal gland nor melatonin appears to affect hormone secretion and fetal growth in pregnant rats.

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