Maternal melatonin influences rates of somatic and reproductive organs postnatal development of male rat offspring

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Abstract

Female rat dams, housed in 12L:12D photoperiod, were pinealectomized or injected daily 1½ h before onset of darkness with 250 μg melatonin/100 g BW, during pregnancy; control and pinealectomized dams received a placebo. Somatic, reproductive organs and gonadotropins levels luteinizing hormone (LH) and follicle stimulating hormone (FSH) of male offspring were examined at the following phases of their sexual development: neonate, infantile, juvenile or prepubertal and pubertal periods. Pinealectomy of the mother produced an altered developmental pattern in the offspring (PIN-X offspring). During the infantile period when pups are lacking maternal melatonin and their own melatonin rhythm is not yet established, a delayed growth of body and testis weights was observed. After the second week of life, from 15 to 25 days of age, coinciding with the initiation of the melatonin rhythm, a speed-up growth of body and testes was observed, followed by a delayed growth from 25 to 30 days, in the juvenile period; this also coinciding with reduced LH levels observed at 30 days of age. Indeed, in PIN-X offspring significantly greater growth rate was observed during the pubertal period than in control offspring, which could be due to the increase in LH secretion up to normal values observed in the PIN-X offspring. Seminal vesicles of the PIN-X offspring also showed delayed growth, which was overcome at the pubertal period. Melatonin (MEL) treatment during pregnancy produced minor alterations in postnatal development of the reproductive tract. Only increased pituitary gland weight was observed at 15 and decreased at 25 days of age. At 25 days of age, MEL offspring reached the highest LH values, and at 30 days of age, PIN-X offspring still show low values. Which suggests that other factors than the endocrine activity of the gland are affecting the somatic growth of the pituitary gland. Seminal vesicles weight was delayed at 25 days of age in the MEL offspring. These results indicate that maternal melatonin is necessary for a normal somatic growth and postnatal development of reproductive organs of the offspring.
Introduction

In mammals, the development of the reproductive function starts during intrauterine life (Chiapa and Fink 1977; Dubois 1985). Since the discovery that melatonin crosses the placental barrier in rats (Klein 1972), non-human primates (Reppert et al. 1979) or sheep (Houghton et al. 1993), the possible influence of maternal melatonin on offspring postnatal development has been studied by several authors.

Information of photoperiod is given to the organism through duration of nocturnal melatonin peak (Reiter 1974). The response of male pups of montane vole (Microtus montanus) to the intermediate photoperiod of 14h light/dark may be different depending on the longer or shorter photoperiod to which their mothers were exposed during pregnancy. Differences in body weight, total length and reproductive tract weight after weaning between voles exposed to prenatal short or long photoperiods and raised by foster mothers have demonstrated that these differences can be attributed to factors acting in utero rather than on maternal effects during lactation (Horton 1985). A similar response about the prenatal photoperiod on regulation of rates of pubertal testicular development (Stetson et al. 1989) or testicular and uterine weights (Horton et al. 1990) have been observed in Siberian hamsters (Phodopus sungorus). The maternal pineal gland and its hormone melatonin are involved in this process through the transmission of photoperiodic information (Weaver and Reppert 1986; Elliot and Goldman 1989; Horton et al. 1989; Lee et al. 1989; Stetson et al. 1989; Jarrige et al. 1987, 1992).

Participation of maternal pineal gland in the regulation of rat reproductive function of male offspring has also been described. Jarrige et al. (1987) found that testicular function of the rat after exposure of the mother to a short photoperiod beginning before conception is decreased by maternal pinealectomy. Jarrige et al. (1992) also found a stimulatory effect of short photoperiod in testicular activity in comparison to offspring raised in long photoperiod. This stimulatory effect was not dependent on the off-spring's own pineal gland since it was observed in both sham-operated and neonatally pinealectomized rats.

Recently, we have found (Colmenero et al. 1991) that melatonin treatment during gestation delayed the sexual maturation of the female offspring. However, the effect of the mothers’ pineal gland in offspring development is still poorly understood. In the present study, we have examined the somatic and reproductive tract growth throughout sexual development in male rat offspring when pregnant mothers were lacking melatonin secretion due to pinealectomy of the mother or exposed to higher melatonin levels by melatonin treatment during pregnancy. The possible influence of hormones with gonadal action as LH and FSH were also evaluated in order to check whether the modifications could be attributed to changes in gonadotropin levels.

Material and Methods

Animals

Female Wistar rats from our colony and weighing 170-250 g at the beginning of the experiment were used. The animals were housed under a 12h light/dark cycle (lights on at 24.00h) and at room temperature of approximately 23°C, with standard rat chow and water ad libitum. After a 3 week period of acclimatization to artificial days, the animals were divided into three groups: control (N=15), pinealectomized (PIN-X; N=11) and melatonin (MEL) treated mother rats (N=15). Mating pairs were held in polypropylene cages, one male with two females. Possible pregnancy was monitored by the presence of vaginal spermatozoa. After mating, females were placed one per cage.

At delivery, litter composition and number of pups were recorded. In order to get uniformity in the development of the pups, on the day of birth each litter was adjusted to 12 pups per dam by cross-fostering some pups from larger litters within treatment groups. Pups remained with the mother until weaning on day 21 (birth=day 0). To study male offspring we followed the classification of Ojeda et al. (1980) concerning postnatal maturation: neonatal period (between birth and day 7 of life, animals being examined on day 5); infantile period (between days 8 and 21, animals being examined on day 15); juvenile or prepubertal period (extends from day 21 to day 35, animals being examined on days 25 and 30); pubertal period for male offspring (day 35 to days 55-60, animals being examined on day 55). Pups were decapitated at either of these five different stages, 5.5h after lights off, under dim red light. Blood was centrifuged and plasma was stored frozen at -20°C until assayed for LH and FSH. Each litter, sacrificed on day 5, was used to obtain one pool of blood. Body, pituitary gland, testis and seminal vesicle weights were recorded at each time point. In the pituitary gland, adenohypophyses and neurohypophyses were both dissected. Coagulating gland was discarded from seminal vesicles.

Melatonin treatment

Melatonin (Sigma Chemical Co) was dissolved in a small volume of absolute ethanol and then diluted in 0.9% NaCl to a dose of 250µg/100 g body wt. This dose was used based on previous findings (Klein 1972) in which 20 µCi of ³H-acetyl-melatonin was administrated to pregnant rats, and that each fetus contained slightly more than 0.1% (20 µCi). Melatonin treatment was given by s.c. injection at the end of the light phase to produce inhibition of the
reproductive system (Reiter et al. 1976; Tamarkin et al. 1976) and daily throughout gestation. Control and PIN-X mother rats received ethanol/saline alone.

Experimental procedures

Pinealectomy was carried out according to the procedure described by Pérez-Casas et al. (1979). Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg body wt); a longitudinal cut was made 2 cm in front to 1 cm behind the inion, and the epicranial aponeurosis was cut and the periostium was removed from the skull between both temporal lines. Using a cylindrical trephine powered by a dentist’s drill, a circular bone disc was cut centered on the lambda point and removed, after which incision of the dura mater was made, with the aid of an ophthalmal scalpel along the lower edge of both lateral sinuses. The right transverse sinus was then cut after halting blood circulation by electrocoagulation with a bipolar electrode. The procedure allowed us to remove the pineal gland and immediately to replace the dura mater and the bone disc. With this surgical procedure the venous return was not subjected to any disturbances because blood was able to flow through the intact left sinus to the jugular vein. The survival rate of the animals was greater than 80%. After surgery, the animals were allowed to recover for 15 days.

Radioimmunoassays of gonadotropins

Plasma LH and FSH levels were measured by specific RIAs employing second antibody-facilitated separation and reagents kindly donated by the National Institute of Health (NIADDK, Bethesda, MD). The assays were validated in our laboratory. Values of LH concentrations were expressed as pg/ml in terms of NIADDK-rat LH-PR-3. The sensitivity of the assay was 20 pg LH/ml. The final dilution of anti-rat LH was 1:100,000. Values of FSH were expressed in ng/ml of FSH-RP-3; the sensitivity of the assay being 12.5 ng FSH/ml. The final dilution of anti-rat FSH was 1:2,000. All samples were measured in the same assay in order to avoid interassay variation.

Statistical analysis

Statistical analysis was performed using the SIGMA Statistics program (Copyright Horus Hardwarer, 1986). Results were expressed as mean ± SEM. Comparisons of body and organ weights among groups at each time point studied were determined by one-way analysis of variance (ANOVA); individual comparisons were then made by Neuman-Keuls multirange test. A longitudinal examination of gonadotropins mean blood levels over time in each group was also carried out by ANOVA.

Results

Body weight increased in all groups throughout the different stages of sexual development (Table 1). Pin-X offspring showed significantly higher body weights at 5 (p<0.05) and 55 (p<0.01) days of age, and significantly lower (p<0.05) at 30 days of age as compared to the control offspring. These differences affected the developmental pattern of body weight gain in PIN-X offspring (Fig. 1). During the infantile period (5-15 days of age) significantly lower (p<0.05) weight gain was observed as compared to control offspring. But during the first part of the juvenile period (15-25 days of age) significantly higher and during the last part of the juvenile period (25-30 days of age) significantly lower (p<0.01) than in the other two groups studied. During the pubertal period (30-55 days of age), again PIN-X offspring, showed significantly higher (p<0.01) weight gain as compared to the other two groups studied as a consequence of the lower body weight shown at 30 days of age. Control offspring also showed significantly lower body weight gain than MEL offspring during the pubertal period. The pituitary gland weight (Table 2) of 15-day-old

<table>
<thead>
<tr>
<th>Table 1. Effect of pinealectomy or melatonin treatment (250 μg/100 g BW.) during pregnancy on body weight of male rat offspring.</th>
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<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
</tr>
<tr>
<td><strong>CONTROL (A)</strong></td>
</tr>
<tr>
<td><strong>PIN-X (B)</strong></td>
</tr>
<tr>
<td><strong>MELATONIN (C)</strong></td>
</tr>
<tr>
<td>0 days</td>
</tr>
<tr>
<td>5 days</td>
</tr>
<tr>
<td>15 days</td>
</tr>
<tr>
<td>25 days</td>
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<tr>
<td>30 days</td>
</tr>
<tr>
<td>55 days</td>
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</tbody>
</table>

Values are mean ± SEM. Numbers in parentheses are number of cases.
5 days: *p<0.05 vs. A; 25 days: *p<0.01 vs. C; 30 days: *p<0.05 vs. A; **p<0.05 vs. C; 55 days: *p<0.05 vs. A.
rats was significantly higher in PIN-X or MEL offspring (p < 0.05; p < 0.01) than in control offspring. However, in MEL offspring pituitary weight at 25 days of age was significantly lower (p < 0.05) than in control offspring. The pituitary weight gain (Fig. 1) in the experimental groups showed different patterns of development until the beginning of the juvenile period as compared with control offspring. Pituitary weight gain of control offspring during the infantile period (5 to 15 days of age) was significantly lower (p < 0.01) than in the experimental groups. For control offspring, the significantly increased (p < 0.01) pituitary weight gain was delayed until 15 to 25 days of age. No significant differences were observed at the end of the juvenile period. In the pubertal period, from 30 to 55 days of age, significantly increased (p < 0.01) pituitary weight gain was observed in MEL offspring as compared to the other two groups.

Development of testis weight (Table 3). Melatonin treatment of the mother did not affect testicular development of the offspring. However it was altered in PIN-X offspring until the beginning of the juvenile period. During the neonatal period, 5-day-old rats, testis weight showed significantly higher values (p < 0.05; p < 0.01) than in control or MEL offspring. This effect was reversed at 15 days of age, showing significantly lower (p < 0.01) testis weight than control offspring. At 25 days of age, testis weight was significantly higher (p < 0.01) in PIN-X offspring as compared to the other two groups. Differences in testis weight are reflected on weight gain (Fig. 3). PIN-X offspring showed significantly lower weight gain from 5 to 15 days of age (p < 0.05) than in control offspring; significantly higher (p < 0.01) from 15 to 25 days of age and significantly lower (p < 0.05; p < 0.01) from 25-30 days of age as compared to the other two groups. From 30 to 55 days of age, PIN-X offspring weight gain was significantly higher than in control offspring.

Seminal vesicles were weighed throughout the sexual development (Table 4). In control offspring, significantly higher (p < 0.05; p < 0.01) weight was observed at 25 days of age as compared to the experimental groups. This delay was overcome at 30 days of age in MEL offspring, but still remained in the PIN-X offspring, with significantly lower (p < 0.05) values than in MEL offspring. Weight gain of seminal vesicles is shown in Fig. 4. In control offspring significantly higher (p < 0.05) weight gain from 15 to 25 days of age was observed as compared to the experimental groups. Significantly lower weight gain was observed from 25-30 days of age in control (p < 0.05)

![Fig. 1. Body weight gain (g) of male rat offspring of control, pinealectomized (PIN-X) and melatonin treated (250 µg/100 g BW.) mother rats.](image)

5-15 days: ** p<0.05 B vs. A.
15-25 days: * p<0.01 B vs. A and C.
25-30 days: * p<0.01 B vs. A and C vs. A.
30-55 days: * p<0.01 B vs. A and C;
** p<0.05 A vs. C.

### Table 2. Effect of pinealectomy or melatonin treatment (250 µg/100 g BW.) during pregnancy on the weight of pituitary glands from male rat offspring.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>CONTROL (A)</th>
<th>PIN-X (B)</th>
<th>MELATONIN (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.67 ± 0.04 (24)</td>
<td>0.72 ± 0.06 (13)</td>
<td>0.74 ± 0.04 (22)</td>
</tr>
<tr>
<td>15</td>
<td>0.80 ± 0.06 (21)</td>
<td>1.37 ± 0.07 (18)*</td>
<td>1.14 ± 0.11 (17)**</td>
</tr>
<tr>
<td>25</td>
<td>3.05 ± 0.31 (15)</td>
<td>2.73 ± 0.21 (16)</td>
<td>2.16 ± 0.08 (17)*</td>
</tr>
<tr>
<td>30</td>
<td>5.09 ± 0.82 (13)</td>
<td>4.98 ± 0.70 (13)</td>
<td>3.51 ± 0.39 (10)</td>
</tr>
<tr>
<td>55</td>
<td>9.04 ± 0.92 (23)</td>
<td>11.49 ± 1.07 (10)</td>
<td>10.02 ± 1.61 (15)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Numbers in parentheses are the number of cases.
15 days: *p<0.01 vs. A; **p<0.05 vs. A. 25 days: *p<0.05 vs. A.
and PIN-X offspring (p<0.01) than in MEL offspring. As it was observed in the other structures studied, PIN-X offspring showed significantly higher (p<0.05; p<0.01) weight gain from 30 to 55 days of age as compared to the other two groups.

The evolutive secretion pattern of LH (Fig. 5) in control offspring showed significantly increased (p<0.05) plasma LH values from 25 to 30 days of age, having these time points significant differences (p<0.05; p<0.01) with data obtained during neonatal and infantile periods. Then values decreased at 55 days of age but were significantly higher than at 5 and 15 days of age. PIN-X offspring showed lower LH values than in the other two groups studied. LH values at 25 days of age were significantly higher (p<0.01) than values from neonatal and infantile periods as was found in control offspring. However, no significant differences were observed between 25 and

<table>
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<th>Table 3. Effect of pinealectomy or melatonin treatment (250 μg/l 100 g BW.) during pregnancy on testis weight of male rat offspring.</th>
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<tbody>
<tr>
<td><strong>CONTROL (A)</strong></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>0 days</td>
</tr>
<tr>
<td>5 days</td>
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<tr>
<td>15 days</td>
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<tr>
<td>25 days</td>
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<tr>
<td>30 days</td>
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<tr>
<td>55 days</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Numbers in parentheses are the number of cases.

5 days: *p<0.05 vs. B; **p<0.01 vs. B.
15 days: *p<0.01 vs. B.
25 days: *p<0.01 vs. A and C.

Figure 3. Testis weight gain (mg) of male rat offspring of control, PIN-X and melatonin treated (250 μg/100 g BW.) mother rats.

5-15 days: *p<0.01 A vs. B.
15-25 days: *p<0.01 A vs. B and C.
25-30 days: *p<0.01 C vs. B;
**p<0.05 B vs. A.
30-55 days: **p<0.05 A vs. B.
Fig. 4. Seminal vesicle weight gain (mg) of male rat offspring of control, PIN-X and melatonin treated (250 µg/100 g BW.) mother rats.

15-25 days: p<0.01 A vs. B and C.
25-30 days: ** p<0.05 C vs. A; * p<0.01 C vs B.
30-55 days: p<0.01 B vs. A and C; ** p<0.05 A vs. C.

Discussion

The data presented here show that the lack of maternal melatonin during pregnancy, produced by pinealectomy of the mother, determined a different rhythm of growth during the infantile and juvenile periods of the sexual development, this being reflected in the fluctuations in the rates of body weight gain. Alterations of body weight observed between 15 and 25 days of age, resulted in increased body weight gain at 25 days of age at the time when endogenous melatonin rhythm of the animal starts to function, after the second week of life both in hamsters (Tamarkin et al. 1980) and in rats (Tang and Pang 1988; Velazquez et al. 1992). These variations suggest the possibility of a role of maternal melatonin as necessary for a normal somatic growth, which was also suggested by the delayed body weight growth observed in offspring of pinealectomized mother rats from 5
Maternal melatonin influences rates of somatic and reproductive organs postnatal development of male rat offspring
to 15 days of age. At that age, suckling pups receive melatonin from the mother’s milk (Reppert and Klein 1978; Velazquez et al. 1992). Similarly, in voles (Microtus montanus) somatic development of pups was increased (Lee et al. 1989) when melatonin (10 µg/day) was administrated to the mothers during the period of lactation. These effects indicate a facilitating role of maternal melatonin on growth during the prepubertal period. In this way at 30 days of age, significantly lower body weight was observed in male PIN-X offspring as it was previously found in female offspring (Diaz et al. 1995). On the other hand, prenatal melatonin treatment did not cause major alterations on the postnatal body weight of male offspring. About this matter, melatonin treatment (0.2 mg/kg b.w.) to prepubertal boys resulted in decreased plasma GH levels (Lissoni et al. 1986). In blind rats or normal rats kept in constant darkness, reduced body weight, tibial length and pituitary GH content were observed (Smith and Lazarus 1973; Sorrentino et al. 1971). However, hamsters treated with melatonin injections showed increased body weight (Tamarkin et al. 1976; Reiter et al. 1977) and increased IGF-I and GH levels (Vriend et al. 1990).

The developmental pattern of the pituitary gland in the control group is an index of the functional state of the gland during sexual maturation. Our results are according to data in the literature indicating that pituitary FSH content remains at low levels until day 17 of age and pituitary LH content starts to increase between 27-33 days of age (Döhler et al. 1977). However, the developmental pattern was affected by both prenatal melatonin treatment or pinealectomy of the mother. The increased pituitary weight observed in both experimental groups at 15 days of age, produced significantly lower pituitary gland weight gain at 15-25 days of age as compared to control group. These differences disappeared at the end of juvenile period at 30 days of age, when most of neuroendocrine processes involved in sexual maturation were developed (Ketelsleger et al. 1978; Chan et al. 1981; Dalkin et al. 1981). No increased gonadotropin levels were observed at 15 days of age. Indeed, at 25 days of age, MEL offspring reached the highest LH values, and at 30 days of age PIN-X offspring still show low LH values. All these data suggest that other factors than the endocrine activity of the gland.
are affecting the somatic growth of the pituitary gland. Again, in PIN-X offspring the pituitary weight showed significantly higher gain values during the pubertal period than in the other two groups studied, which again suggest that maternal melatonin is necessary for a normal growth of the offspring.

In reference to the development of the sexual organs, testes and seminal vesicles, a greater rate of growth was observed during the juvenile period as compared to the neonatal and infantile periods. Seminal vesicles development was slower than testis growth. The growth rate observed from 25 to 30 days of age in the three groups studied represent the sexual maturation process of the neuroendocrine mechanisms at the end of juvenile period of male rats (Ketelsleger et al. 1978). Testis weight development did not show modifications in MEL offspring. However, in PIN-X offspring, differences were observed at 15 and 25 days of age with reduced testis growth during the infantile period, and recrudescent growth at 25 days of age, that produced significantly greater testis weight gain at 25 days of age as was observed for body weight. After this age, no significant differences were observed in testis weight. In agreement with our results, previous findings in the Syrian hamster (Elliot and Goldman 1989) have shown delayed testicular development in 34-day-old offspring of pinealectomized mother hamsters, and differences disappeared at 55 days of age. In male rats, no significant differences were observed in both testes and seminal vesicles in 30-, 42- and 49-day-old offspring of pinealectomized mother hamsters, and differences disappeared at 55 days of age. In male rats, no significant differences were observed in testis weight. In agreement with our results, previous findings in the Syrian hamster (Elliot and Goldman 1989) have shown delayed testicular development in 34-day-old offspring of pinealectomized mother hamsters, and differences disappeared at 55 days of age. In 30-day-old male rats, increased LH secretion was observed in all groups studied except in PIN-X offspring that still show low values. This LH impairment could be involved in the reduced development of the sexual organs observed at that age. Having in mind that modification of endocrine parameters during sexual development in male rats is characterized by small changes in plasma LH secretion (Debeljuk et al. 1972). In all groups studied, the greater testis growth rate was observed during the pubertal period from 35 to 55-60 days of age, which is in agreement with data in the literature. During the pubertal period the sexual organs of the rat reach adult size, free spermatides are observed at 45 days of age in tubular lumen and at 55-60 days of age in different vessels (Clegg 1960; Ketelsleger et al. 1978; Payne et al. 1977). Indeed, in PIN-X offspring significantly greater growth rate was observed during the pubertal period than in control offspring, which could be due to the increase in LH secretion up to normal values in PIN-X offspring. Our results indicate that maternal melatonin is necessary for a normal sexual development, but this influence is overcome during the juvenile period when melatonin rhythm is established.

Acknowledgments

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REFERENCES

Chiappa SA and Fink G (1977) Endocrinology 72: 211
Clegg EJ (1960) J Reprod Fertil, 1: 118
Debeljuk L, Arimura A, Schally AV (1972) Endocrinology 90: 585
Dubois PM (1985) Endocrinology 116: 1565
Horton TH (1985) Biol Reprod 33: 934
Houghton DC, Walker DW, Young IR, McMillen IC (1993) Endocrinology 13: 90
Ketelsleger JM, Hetzel WD, Sherius RJ and Catt KJ (1978) Endocrinology 103: 212
Klein DC (1972) Nature 237: 117
Pérez-Casas A, Villa Vigil A, Bengoechea ME (1979) Congresus Europensis Prague, Argumenta Communicatorum 313
Reppert SM and Klein DC (1978) Endocrinology 102: 582
Smithe GA and Lazarus L (1973) Nature 244: 230
Weaver DR and Reppert SM (1986) Endocrinology 119: 2861