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Effect of prenatal melatonin exposure on gonadotropins and prolactin secretion in male and female rat pups

Yuji Okatani [a,*], Akihiko Wakatsuki [a], Edet. E. Otukonyong [b], Yasuyo Miyahara [a]

[a] *Department of Obstetrics and Gynecology, Kochi Medical School, Oko, Nankoku, Kochi 783-8505, Japan*
[b] *Department of Physiology, Kochi Medical School, Kochi 783-8505, Japan*

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Abstract

We evaluated whether melatonin administration to pregnant rats during the final week of pregnancy affects prepubertal secretion of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin in offspring. Melatonin was administered in the drinking water from day 14 to delivery. LH, FSH and prolactin concentrations were determined in plasma sampled from offspring between 5 and 30 days in the dark portion of the diurnal cycle. Administration of 2 or 20 μg/ml melatonin did not affect LH or FSH in male or female offspring. The 20-μg/ml dose caused a significant increase in prolactin in males and females at day 15. In contrast, melatonin, 2 or 20 μg/ml, decreased prolactin at days 25 and 30 in females and day 25 in males. Thus, prenatal melatonin exposure alters prolactin secretion, but not that of LH and FSH in infantile and prepubertal male and female rats. © 2001 Published by Elsevier Science B.V.

Keywords: Melatonin; Gonadotropin; Prolactin; Pregnancy; Puberty

1. Introduction

Melatonin secreted by the pineal gland contributes to the regulation of biologic rhythms and neuroendocrine function, including reproductive and adrenal interactions (Reiter, 1991). Multiple lines of evidence indicate that melatonin is a powerful scavenger of oxygen free radicals, quenching hydroxyl radicals (Tan et al., 1993; Matuszek et al., 1997) and peroxyl radicals (Pieri et al. 1994) as well as the highly toxic peroxynitrite anion (Cuzzocrea et al., 1997; Okatani et al., 1999), formed by the reaction between superoxide and nitric oxide. In addition to having direct antioxidant activity, melatonin increases the activity of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Pablos et al., 1997; Antolin et al., 1996; Kotler et al., 1998).

Approximately 10% of complications occurring in pregnancy are attributable to preeclampsia, which is a leading cause of maternal and neonatal death worldwide. While the cause of preeclampsia is not known, free radical-induced lipid peroxidation has been suggested as an etiologic factor (Hubel et al., 1989). Further, oxygen free radicals play an important pathogenetic role in perinatal encephalopathy induced by hypoxia and ischemia, which represents a major cause of childhood neurological disability (Mishra and Delivoria-Papadopoulos, 1989).

We previously demonstrated that melatonin suppresses the vasoconstrictive effect of hydrogen peroxide and oxidized low-density lipoprotein in human umbilical artery in an ex vivo preparation, possibly as a result of its ability to scavenge hydroxyl radicals (Okatani et al., 1997, 2000a). Furthermore, we found that melatonin protects against ischemia and reperfusion-induced oxidative lipid and DNA damage in fetal rat brain (Wakatsuki et al., 1999). These biologic actions of melatonin may have clinical implications for the treatment of prenatal conditions in which excessive free radical production has been implicated, such as preeclampsia and fetal hypoxia. However, concern exists because melatonin has been found to be readily transferred at a rapid rate from the maternal to the fetal circulation in both humans and rats (Okatani et al., 1998, 1999, 2000b). This maternal-to-fetal transfer has been reported to produce diurnal variation in melatonin concentration in the umbilical circulation (Munoz-Hoyos et al., 1992; Jaldo-Alba et al., 1993).

* Corresponding author. Tel.: +81-88-880-2382; fax: +81-88-880-2384.
E-mail address: okataniy@med.kochi-ms.ac.jp (Y. Okatani).

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The potential for developmental toxicity to reproductive organs from repeated oral doses of melatonin during gestation has not been evaluated. Melatonin is sold as a dietary supplement in the US under the Dietary Supplement Health and Education Act of 1994. No developmental and reproductive toxicity studies performed in accordance with current regulatory guidelines have been published. The present study was undertaken to evaluate these effects of oral doses of melatonin in rats during the final week of pregnancy, in which preeclampsia typically occurs in humans, in terms of secretion of gonadotropins and prolactin in male and female offspring.

2. Materials and methods

2.1. Animals and treatments

Female Sprague-Dawley rats weighing 200 to 225 g were housed under controlled temperatures (22 ± 1 °C) and a 14-h/10-h light–dark cycle (dark from 11:00 to 21:00 h). All research protocols were approved by the Animal Research Committee of the Kochi Medical School. A commercial solid diet, and drinking water as described below, were provided ad libitum. After a 3-week period of acclimatization to the reversed light–dark cycle, female rats were mated. Day 1 of pregnancy was confirmed by the presence of a vaginal plug.

On day 14 of gestation, the pregnant rats were divided randomly into three groups, two receiving melatonin. Melatonin (Aldrich, Milwaukee, WI, US) was dissolved in a minimal volume of ethanol and then diluted with water to 2 μg/ml for one group and 20 μg/ml for the other. The final concentration of ethanol in these solutions was below 0.5%. Melatonin (2 or 20 μg/ml) administration in the drinking water was initiated on day 14. The third group (control animals) received ethanol/water alone from day 14. Oral administration of melatonin in the drinking water was chosen because continuous high blood concentrations are required to produce an antioxidant effect. Water bottles containing melatonin were covered with aluminium foil to protect the solution from light, and a fresh drinking water solution was provided three times weekly. The volume of daily melatonin-containing water intake per rat was 50.6 ± 2.3 ml (range, 39.1–63.0 ml; $n = 16$).

To obtain greater uniformity in the development of the pups, each litter was adjusted to 10 pups per dam on the day of birth. Pups remained with the mother until weaning on day 21 (birth = day 0). We followed the classification of Ojeda et al. (1980) concerning postnatal maturation: neonatal period (between days 0 and 7 of life; animals were examined on day 5); infantile period (between days 8 and 21; animals examined on day 15); juvenile or prepubertal period (between days 22 and 35; animals examined on day 25 or 30). At each of these time points, pups were killed at 16:00 h, the middle of the dark phase. At 5 days of age, two blood samples were pooled to constitute one sample. Blood was centrifuged, and plasma was stored frozen at −80 °C until assay for luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin. In 30-day-old rats, testes and ovaries were removed and weighed.

2.2. Radioimmunoassays of LH, FSH and prolactin

Plasma concentrations of LH, FSH and prolactin were measured in duplicate using double-antibody radioimmunoassay kits provided by the Pituitary Hormone Distribution Program of the National Institute of Diabetes and Digestive and Kidney Diseases. The assays were validated previously in our laboratory. The final dilution of anti-rat LH, anti-rat FSH and anti-rat prolactin antibodies were 1:750,000, 1:125,000 and 1:437,500, respectively. Plasma concentrations of LH, FSH and prolactin were expressed as picograms per milliliter for NIADDK rat LH-RP-3, nanograms per milliliter for FSH-RP-2 and prolactin RP-3. Sensitivities of LH, FSH and prolactin assays were 100 pg/ml, 0.5 ng/ml, and 1 ng/ml, respectively. Inter- and intra-assay variation was less than 10%.

2.3. Statistical analysis

Data are expressed as the means ± S.E.M., with the number of animals indicated as n. Changes in the pattern of LH, FSH, and prolactin in each group were analyzed by one-way analysis of variance (ANOVA). Mean blood levels at each time point studied were compared between groups by one-way ANOVA. If ANOVA showed a significant change or a significant difference, a multiple-comparison procedure (Scheffe's test) was applied to determine which groups differed. A difference associated with a value of $P < 0.05$ was accepted as statistically significant.

3. Results

The effects of melatonin treatment on body weight and weights of ovaries or testes are summarized in Table 1. No differences in body weight or weight of ovaries or testes in offspring were found among the groups studied.

3.1. Female offspring

Analysis of variance indicated significant age effects in the secretion of LH ($F = 5.328$, $P < 0.01$), FSH ($F = 87.619$, $P < 0.0001$) and prolactin ($F = 35.643$, $P < 0.0001$). Plasma concentrations of LH and FSH increased markedly from day 5 to day 15 ($P < 0.01$, $P < 0.0001$, respectively), decreased significantly from day 15 to day

Table 1
Body weight and ovary or testis weight at day 30 in offspring of control and melatonin-treated rats

	Day 5	Day 15	Day 25	Day 30	Day 30	
	Body weight (BW, g)				Weight of ovary or testis (mg)	Relative weight of ovary or testis (mg/100 g BW)
Control						
Male	10.38 ± 0.80 (30)	25.00 ± 3.88 (19)	43.86 ± 4.66 (11)	76.44 ± 6.43 (16)	645.8 ± 94.5 (16)	842.4 ± 73.0 (16)
Female	9.66 ± 1.01 (29)	23.03 ± 2.75 (22)	42.06 ± 3.32 (16)	69.41 ± 3.86 (17)	39.7 ± 4.6 (17)	57.6 ± 7.5 (17)
Melatonin (2 μg / ml)						
Male	10.41 ± 2.54 (16)	25.59 ± 4.56 (10)	39.76 ± 2.87 (22)	75.62 ± 9.26 (17)	599.0 ± 83.8 (17)	811.4 ± 169.4 (17)
Female	9.97 ± 2.77 (16)	21.69 ± 3.93 (16)	38.26 ± 1.72 (15)	68.15 ± 8.24 (13)	38.9 ± 7.1 (13)	56.9 ± 6.3 (13)
Melatonin (20 μg / ml)						
Male	9.57 ± 1.06 (33)	24.06 ± 1.09 (18)	44.73 ± 10.16 (16)	74.64 ± 9.51 (13)	632.5 ± 134.2 (13)	798.8 ± 96.7 (13)
Female	9.49 ± 0.96 (27)	22.31 ± 1.20 (26)	41.84 ± 5.76 (18)	69.81 ± 7.09 (16)	38.8 ± 3.8 (16)	52.8 ± 13.5 (16)

Data are expressed as the means ± S.E.M. Numbers of animals in parentheses.

25 ($P < 0.01$, $P < 0.0001$, respectively), and then remained unchanged until day 30 (Figs. 1 and 2). Plasma concentrations of prolactin remained low until day 15, and then increased significantly ($P < 0.0001$, Fig. 3). Administration of melatonin to pregnant rats did not produce significant changes in plasma concentrations of LH and FSH in the female offspring. Plasma concentrations of prolactin on day 15 in the offspring exposed to 20 μg/ml of melatonin were significantly higher than those in the controls ($P < 0.0001$) or in animals exposed to 2 μg/ml melatonin ($P < 0.0001$). However, prenatal exposure to 2 or 20 μg/ml melatonin resulted in significant decreases in plasma concentration of prolactin on day 25 ($P < 0.001$ and $P < 0.001$, respectively) and on day 30 ($P < 0.02$ and $P < 0.0001$, respectively) compared with concentrations in control rats.

3.2. Male offspring

Plasma concentrations of LH and FSH remained low until day 25 and increased significantly from day 25 to day 30 ($P < 0.01$ and $P < 0.0001$, respectively, Figs. 4 and 5). Plasma concentrations of prolactin remained low until day 15 and significantly increased from day 15 to day 25 ($P < 0.0001$, Fig. 6). Administration of melatonin did not

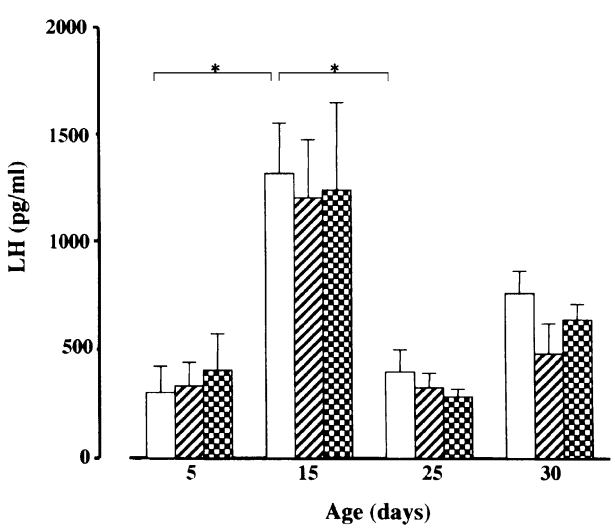

Fig. 1. Age-related changes in plasma concentrations of luteinizing hormone (LH) of female rat offspring in unexposed controls (open columns) and in other animals prenatally exposed to melatonin. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. The symbol "*" indicates a value of P less than 0.01 for the indicated comparison, according to Scheffe's test.

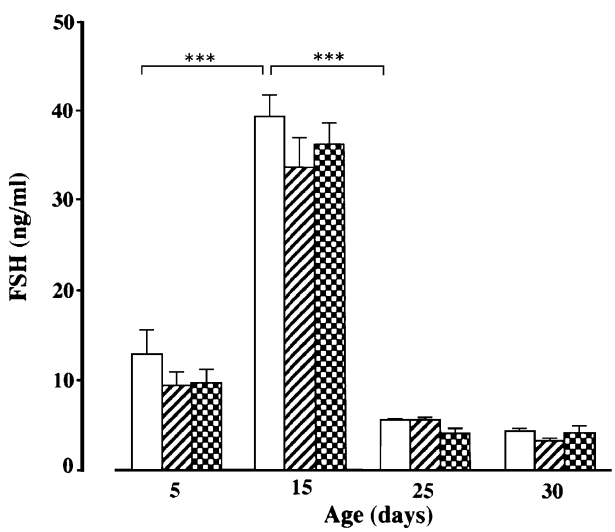

Fig. 2. Age-related changes in plasma concentrations of follicle-stimulating hormone (FSH) of female rat offspring in unexposed controls (open columns) and in other animals prenatally exposed to melatonin. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. The symbol "*" indicates a value of P less than 0.0001 for the indicated comparison, according to Scheffe's test.

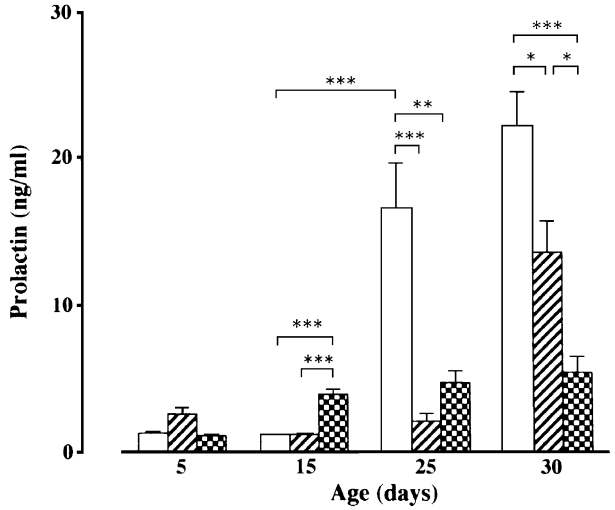

Fig. 3. Age-related changes in plasma concentrations of prolactin of female rat offspring in unexposed controls (open columns) and in other animals prenatally exposed to melatonin. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. $^{*}P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$, obtained by Scheffe's test.

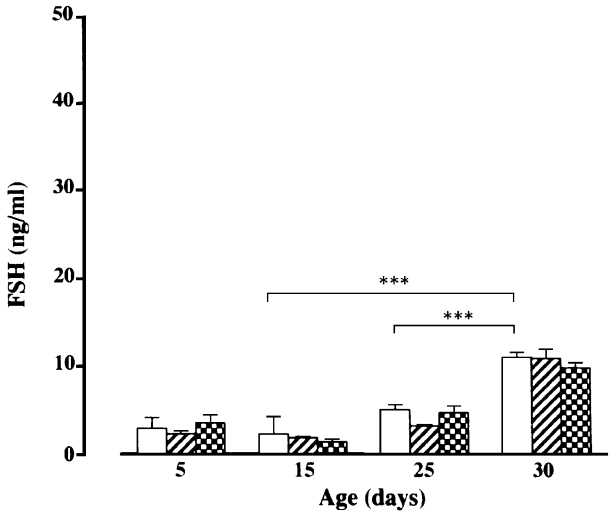

Fig. 5. Age-related changes in plasma concentrations of follicle-stimulating hormone (FSH) of male rat offspring in unexposed controls (open columns) and in other animals prenatally exposed to melatonin. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. $^{*}P < 0.05$, $^{***}P < 0.01$, obtained by Scheffe's test.

affect the plasma concentration of LH and FSH. However, no significant increase in plasma LH at day 30 was found in the melatonin-treated groups. Paralleling changes in female offspring, plasma concentrations of prolactin in animals exposed to 20 μg/ml melatonin were significantly higher than in the control group ($P < 0.001$). Plasma prolactin on day 25 in the groups treated with 2 μg/ml or 20 μg/ml melatonin also was significantly lower than in the control group ($P < 0.0001$ and $P < 0.0001$, respectively). However, at day 30, no significant difference in plasma prolactin was found between the three groups.

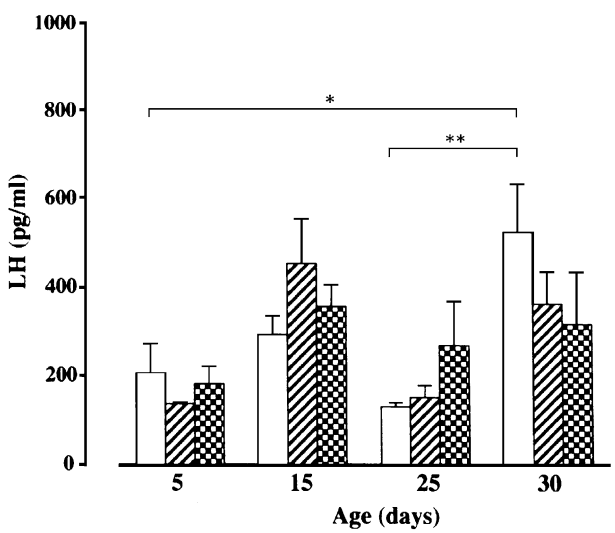

Fig. 4. Age-related changes in plasma concentrations of luteinizing hormone (LH) of male rat offspring in unexposed controls (open columns) and in other animals prenatally exposed to melatonin. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. $^{*}P < 0.05$, $^{**}P < 0.01$, obtained by Scheffe's test.

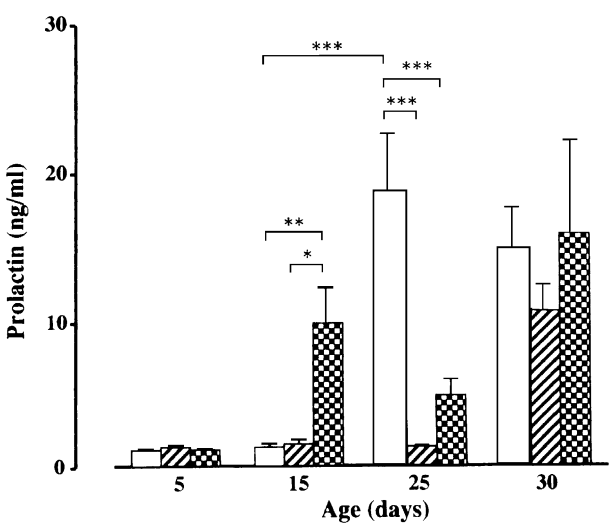

Fig. 6. Age-related changes in plasma concentrations of prolactin of male rat offspring in the untreated control (open columns) and in the prenatally melatonin-treated group. Melatonin at a concentration of 2 μg/ml (diagonally hatched columns) or 20 μg/ml (checkered columns) in drinking water was administered to pregnant rats between day 14 of gestation and the day of delivery. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$, obtained by Scheffe's test.

4. Discussion

Our results show that prenatal oral administration of melatonin during the final week of pregnancy resulted in an altered secretion of prolactin, but not of LH or FSH, in male and female offspring. While few studies have examined the effects of prenatal melatonin exposure on sexual maturation in young rats, Colmenero et al. (1991) and Diaz et al. (1995) reported that maternal administration of melatonin (250 μg/100 g, s.c. per day) throughout gestation resulted in a delay in vaginal opening associated with decreased cellular and nuclear volumes in ovarian oocytes (Fernandez et al., 1995). Other earlier reports suggest that secretion by the maternal pineal gland influences testicular function in male offspring during early life (Jarrige et al., 1990, 1992); androgenic activity in 15-day-old offspring of pregnant rats with brief daily light exposure was higher than in offspring of rats with a long daily light exposure. Moreover, maternal pinealectomy reduced plasma testosterone and dihydrotestosterone concentrations, while daily maternal subcutaneous administration of melatonin (25 μg/day) increased the testicular testosterone content of offspring. Although vaginal opening and testicular function were not examined in the present study, we found no difference in uterine, ovarian or testicular weight. This is consistent with results reported by Colmenero et al. (1991).

Our results are in agreement with previous longitudinal studies of gonadotropins and prolactin in rats from the neonatal period until the prepubertal period (Dohler and Wuttke, 1975; Dohler et al., 1977; Chiappa and Finx, 1976; Payne et al., 1976). While we did not examine the group which received tap water only, the results suggest that the ethanol in the vehicle (below 0.5%) does not influence LH, FSH and prolactin secretion. Plasma concentrations of LH and FSH at 15 days in our control female offspring were significantly higher than those at the other time points; they then declined in the prepubertal period. Plasma LH and FSH in the control male offspring increased significantly at the end of the juvenile period. This elevation of plasma FSH concentration during the juvenile period promotes rapid sexual maturation in male rats (the so-called "FSH period"). We found that melatonin treatment during the final week of pregnancy did not alter postnatal LH and FSH levels in female or male offspring. These findings differ from those of Diaz et al. (1995), who administered melatonin at daily doses of 250 μg/100 g, s.c. at the end of lights-on periods throughout gestation. These authors reported marked treatment-related increases in plasma LH at days 15 and 25 in female offspring, despite the observation of delayed vaginal opening. In male offspring, the authors found a significant increase in plasma LH levels at day 25, but a significant decrease in plasma FSH levels between day 5 and day 55. Testicular function was not examined. The discrepancies between our present findings and those of Diaz et al. (1995) are difficult to reconcile, but they may be related to differences in methods (dose, route, timing, and duration) resulting in different total maternal plasma concentrations of melatonin. Alternatively, they may reflect strain differences. Furthermore, the melatonin-treated groups in the present study did not show the significant increase in plasma LH at day 30 observed in the control male animals. At present, however, it is not clear whether this can delay sexual maturation of male offspring. As far as we know, our study is the first in which circulating hormones of rat offspring were measured following oral administration of melatonin during gestation.

Our most surprising finding involved changes in plasma prolactin concentrations in melatonin-exposed male and female offspring. Changes in plasma prolactin over time in untreated control male and female offspring were consistent with those in previous reports (Dohler and Wuttke, 1975; Dohler et al., 1977). Melatonin exposure resulted in a significant increase in plasma prolactin levels at postnatal day 15 in male and female offspring, but a significant decrease at day 25 in male offspring and at day 25 and day 30 in female offspring. Previous reports suggest physiologic involvement of prolactin in the regulation of prepubertal ovarian and testicular function. Prolactin has been shown to support ovarian maturation by enhancing ovarian responsiveness to gonadotropins (Clemens et al., 1969). Long-term stimulation of endogenous prolactin release by blockade of dopaminergic receptors advances the onset of puberty and increases estradiol and progesterone responses of the ovary to chorionic gonadotropin and FSH (Advis and Ojeda,1978; Advis et al., 1981a). Ovaries from hyperprolactinemic rats exhibit an increased number of LH receptors (Advis et al., 1981b). The relatively low plasma prolactin concentrations demonstrated in the present study during the prepubertal period may be related to the reported delayed maturation of female offspring prenatally exposed to melatonin. Testicular responsiveness to LH also appears to be influenced by prolactin, which shows a progressive increase in plasma during the prepubertal period. Treatment of hypophysectomized rats with prolactin increases their ability to produce testosterone in response to short-term stimulation with exogenous LH (Bartke, 1980). Treatment with prolactin reportedly increases the numbers of LH receptors in the testes of hypophysectomized rats (Zipf et al., 1978). The elevation of plasma prolactin at day 15 may be responsible for the increased androgenic activity reported to occur at day 15 in male rats treated with melatonin during gestation (Jarrige et al., 1992). However, the mechanism by which prenatally administered melatonin alters prolactin secretion of the offspring is not yet known.

Clinically recommended oral doses of melatonin include 0.2–10 mg to induce sleep, 1–10 mg to alleviate jet lag, 0.1–3 mg to modulate effects of aging, 1–5 mg to enhance adjustment to work and 2–20 mg to stimulate immune responses (Reiter and Robinson., 1995). The daily

dose of melatonin in the present study was approximately 0.4–4 mg/kg. Jahnke et al. (1999) have reported that oral administration of daily doses of 1–200 mg/kg during gestation does not affect fetal weight, or the incidence of fetal malformations or anatomic variation. However, further studies of fetal safety are required before melatonin is used in the treatment of prenatal conditions.

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