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EFFECT OF NEONATAL MELATONIN ADMINISTRATION ON SEXUAL DEVELOPMENT IN THE RAT

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Summary—In order to study the mechanisms by which melatonin modulates sexual development, 5-day-old female Wistar rats have been treated with a single s.c. injection of melatonin, 3 h before the darkness onset. Criteria for sexual development were the age of vaginal opening and the circulating levels of prolactin, LH, FSH and estradiol. Also, pineal melatonin content was measured. There was a precocious puberty ($P < 0.01$) in melatonin-treated rats measured by the age of the vaginal opening. An increase in the number of estrous smears over the whole period studied was observed in melatonin-treated animals as compared to controls. Along with these modifications, there was decrease in pineal melatonin content and serum prolactin levels, on day 21 of life ($P < 0.05$), with an increase in both parameters on day 30 of age, in melatonin-treated rats as compared to controls, with no modifications at any other time studied. No differences were detected for serum LH levels considering the whole period studied for both groups. There was a faster decrease in plasma FSH levels with age in melatonin-treated animals than in controls. Serum estradiol levels were decreased in the peripubertal period in melatonin-treated rats as compared to controls. All these data suggest that the modifications induced by neonatal melatonin administration on prolactin, FSH and estradiol could be responsible for the precocious puberty shown in this study.

INTRODUCTION

It is well established that melatonin mediates the effect of the pineal gland on reproduction [1–8]. In the laboratory rat, daily injections of melatonin between 20 and 40 days of age showed a dose-dependent inhibitory effect on reproduction; in contrast, daily injections at earlier ages or in adulthood did not have a significant influence on sexual maturation [9]. The time of administration during photophase seems to be critical for melatonin action [4, 6, 10–13]. It is also known that neonatally rats are very sensitive to different manipulations that modify sexual behaviour in adulthood [14, 15]. All these manipulations involve a single injection, on day 5 of life, of the given pharmacological agent [14].

The objective of the present work was to examine the possible effect of melatonin on the process of sexual maturation when given as a single postnatal injection to rats. Melatonin (100 μg/rat) was injected in female rats, on day 5 of life, 3 h prior to the onset of darkness. Criteria for assessment of sexual development were the time of vaginal opening and the levels of serum FSH, LH, prolactin and estradiol.

MATERIAL AND METHODS

Animals

Thirty Wistar dams were used for this study. Immediately after birth, offspring were counted and randomized to a number of 8–9 pups/dam. They were kept under controlled conditions of light–dark cycles (12:12, lights on at 8 a.m.) and temperature (21°C). They were fed *ad libitum* with Sanders rat chow.

Animals were killed by decapitation and blood was collected from the trunk in glass tubes. After clotting, the samples were centrifuged at 1500 *g* at 4°C, and the serum was kept frozen until analyzed.

Melatonin administration

On day 5 of life, female pups received 100 μg of melatonin or vehicle, subcutaneously, 3 h prior to the onset of darkness. Melatonin was dissolved in a mixture of saline with 1% ethanol. Vehicle without melatonin was administered to the control groups.

Onset of puberty and sexual development

The onset of puberty was controlled by vaginal opening. Also, daily vaginal smears were taken both in melatonin and vehicle-treated rats to study the possible effect of the pineal indoles on the estrous cycle. Groups of 7 or 8 rats were killed on days 21, 30, 35, 40, 45, 50, 60 and 70 of life, to study serum prolactin, LH, FSH and estradiol evolution throughout sexual development. Immediately after killing the animal the pineal gland was removed, placed on dry ice and kept frozen at −70°C until melatonin assays were performed.

Hormonal determinations

Serum prolactin, LH and FSH were measured by specific double antibody RIA systems using reagents kindly supplied by the NIAMDDK (NIH, Bethesda, MD), and previously validated in our laboratory. Prolactin assay had a sensitivity of 0.4 μg/l and intra- and interassay coefficients of variation of 5 and 9%

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respectively. The variability for LH and FSH was described elsewhere [16]. All the samples were measured in the same assay to avoid possible modifications in serum hormone levels due to variation coefficients. Serum estradiol levels were measured using a commercial kit purchased from Sorin, Biomedica, Saluggia (Vercelli, Italy), previously validated in our laboratory. Pineal melatonin content was measured by RIA methodology by using a specific antibody, generously given by Prof. G. M. Brown (McMaster University, Hamilton, Ontario, Canada) and previously validated in our laboratory [17].

Statistics

All data were subject to two-way analysis of variance, and comparison between groups was made through a Mann–Whitney U-test. In some cases a chi-square test was employed.

RESULTS

In Table 1, the age of vaginal opening and estrous cyclicity is shown. Melatonin administration induced precocious puberty as indicated by the advance of the time of vaginal opening as compared to the controls ($P < 0.01$). Melatonin-treated animals showed an increase in the number of estrous smears as compared to controls ($P < 0.05$) whereas the number of diestrous smears were reduced ($P < 0.05$).

In Fig. 1, basal serum prolactin levels are depicted. There is a marked decrease in prolactin concentrations of melatonin-treated rats on day 21 and 60 ($P < 0.05$) together with an increase on days 30, 40 and 50 of age ($P < 0.05$) as compared to vehicle-treated female rats. No differences were apparent at any other time studied.

Table 1. Influence of neonatal melatonin administration on the timing of vaginal opening and estrous cyclicity

Vaginal opening (days)		Estrous cycle (%)			
		Control.		Exp.	
Control.	Exp.	D	E	D	E
36 ± 0.3	34 ± 0.4	60	40	37	63

E—estrous; D—diestrous.
$n = 30$ animals per group.

Serum LH levels are shown in Fig. 2. A decrease in circulating LH levels was observed on days 35 and 45 of age ($P < 0.05$) while no differences were observed at any other interval studied.

For serum FSH, there was a marked decrease in circulating FSH levels in melatonin-treated rats on days 21, 30, 35, 45 and 70 of age ($P < 0.05$) (Fig. 3). No differences were observed at any other studied age.

Figure 4 depicts the serum estradiol concentrations on the same experimental groups. There was a significant decrease on days 21, 40 and 45 of age but no differences could be detected at any other interval in melatonin-treated rats as compared to the vehicle-treated ones ($P < 0.05$).

In Fig. 5 pineal melatonin content is seen. Neonatal melatonin administration resulted in a decrease of pineal melatonin content on day 21 of life together with a significant increase on day 30 as compared to controls.

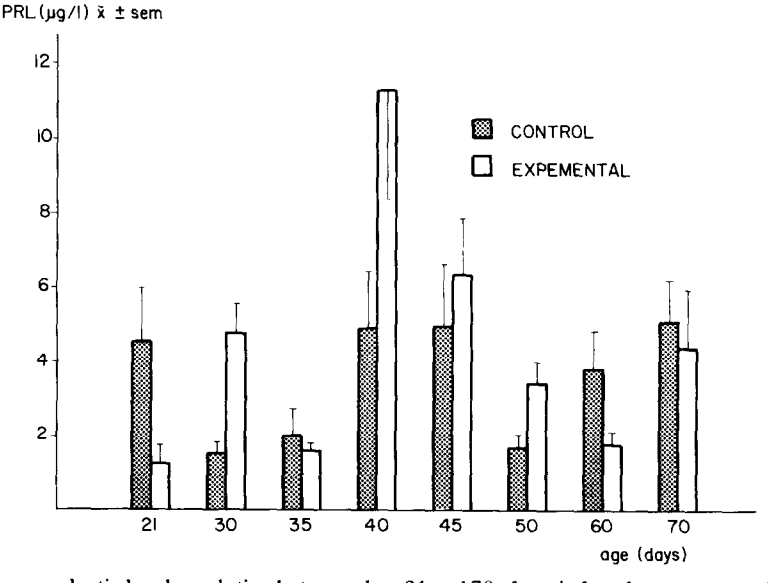

Fig. 1. Plasma prolactin levels evolution between days 21 and 70 of age in female rats, neonatally treated with melatonin (100 µg/rat) or vehicle. Values are expressed as mean ± SEM ($n = 7$–8 animals per group).

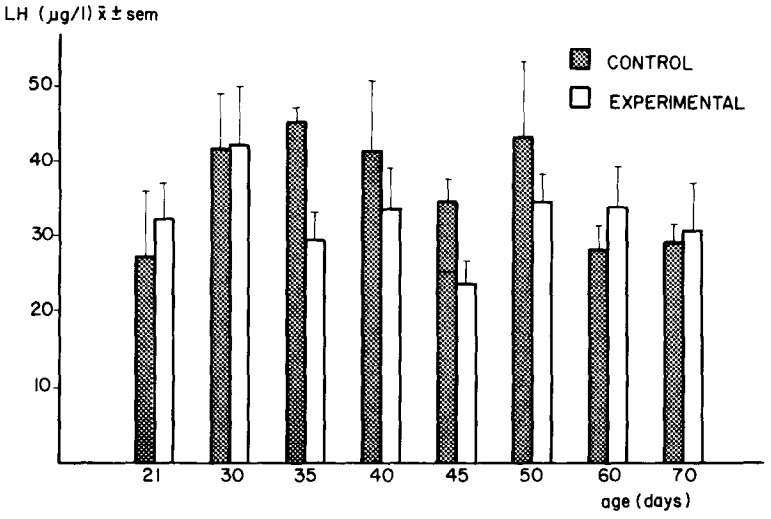

Fig. 2. Plasma LH levels evolution between days 21 and 70 of age in female rats, neonatally treated with melatonin (100 μg/rat) or vehicle. Values are expressed as mean ± SEM (n = 7–8 animals per group).

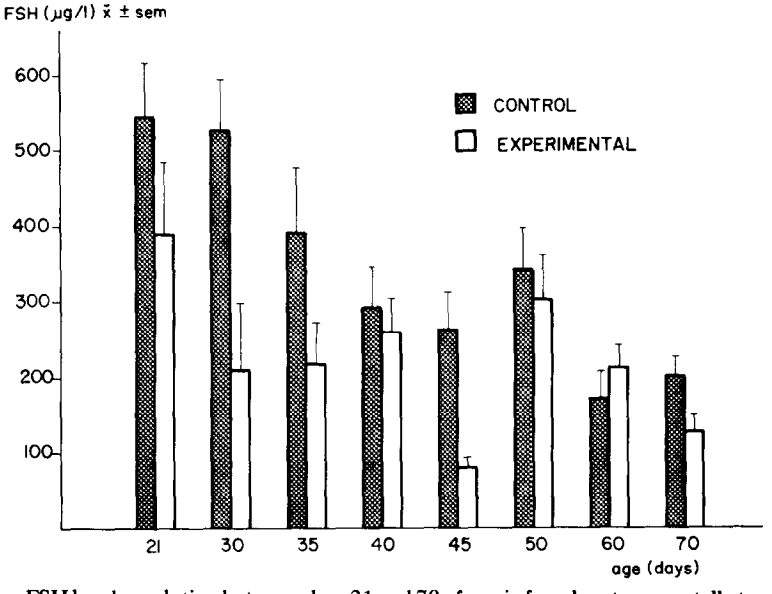

Fig. 3. Plasma FSH levels evolution between days 21 and 70 of age in female rats, neonatally treated with melatonin (100 μg/rat) or vehicle. Values are expressed as mean ± SEM (n = 7–8 animals per group).

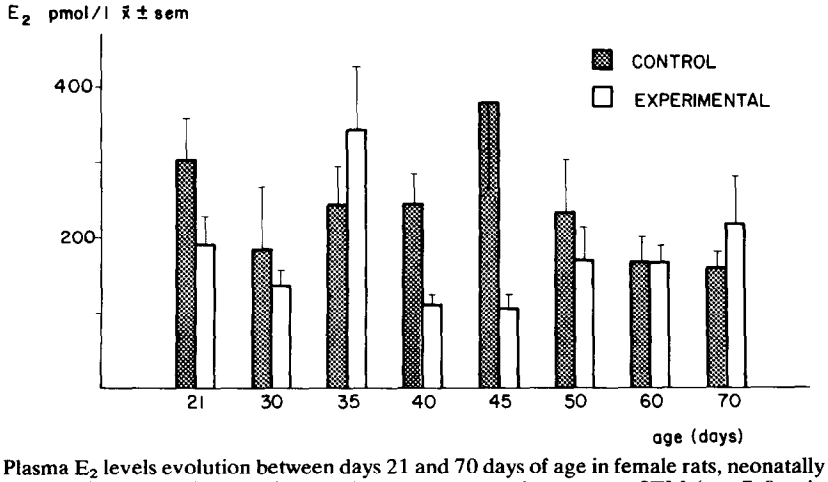

Fig. 4. Plasma E_2 levels evolution between days 21 and 70 days of age in female rats, neonatally treated with melatonin (100 μg/rat) or vehicle. Values are expressed as mean ± SEM (n = 7–8 animals per group).

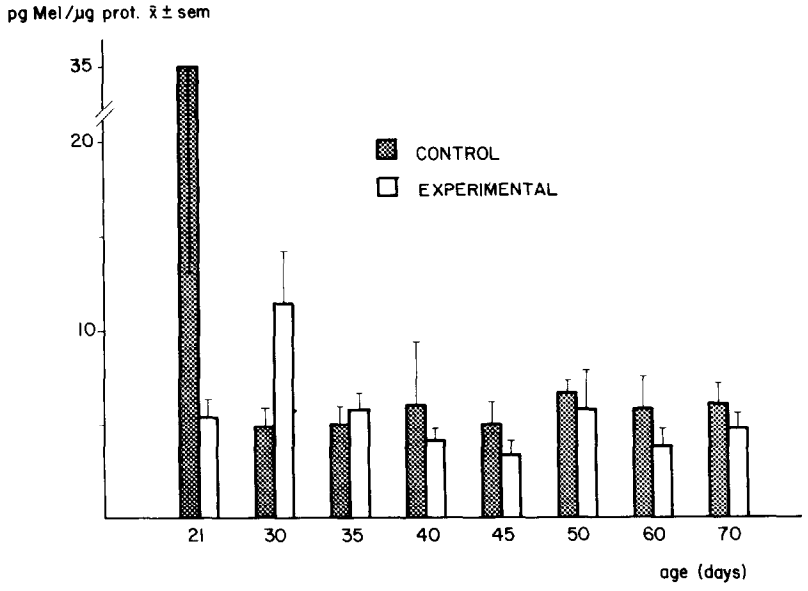

Fig. 5. Pineal melatonin content evolution between days 21 and 70 of age, in female rats, neonatally treated with melatonin (100 µg/rat) or vehicle. Values are expressed as mean ± SEM (n = 7–8 animals per group).

DISCUSSION

The present study demonstrates that a single dose of melatonin affects pubertal development of female rats when given on the 5th postnatal day.

While in the literature there is evidence showing that melatonin exerts an inhibitory role on sexual development depending upon dose [18] and time of the photophase when it is administered [9, 13], our results clearly indicate that neonatal melatonin injection induces precocious puberty in female rats. This treatment also resulted in remarkable modifications of the circulating levels of reproductive hormones (LH, FSH, prolactin and estradiol).

The advance in the age of vaginal opening in melatonin-treated rats can be explained by any or all of the following interpretations:

(a) The marked decrease in pineal melatonin content shown on day 21 could be the signal to begin the pubertal process. These findings would be supported by the observations of Faigon *et al.*[19], who found that pinealectomy in prepubertal animals resulted in a precocious puberty measured by the maturation of estrogen-dependent gonadotropin regulatory mechanisms.

(b) A reduction in serum prolactin levels was found in melatonin-treated female rats on day 21 of age, a finding that may support the occurrence of a precocious puberty. From several previous studies it can be concluded that prolactin exerts an inhibitory role on the reproductive axis [20–23], and that the prolactin decrease in melatonin-treated rats is causally related to the advance of sexual maturation reported above [22].

(c) While no differences in serum LH are found in the two experimental groups examined, in the present work, there was a fast decrease in circulating FSH in melatonin-treated animals is compared to controls. Data from the literature have described that during the pubertal process there is a decrease in FSH to a greater extent than in LH levels [23]. The fast decrease in FSH suggests that puberty has begun earlier in melatonin-treated than in control rats.

The increase in the number of estrous smears is strongly indicative that the disturbances of cyclicity observed resemble those found in neonatally estrogenized rats [14, 15], in which a continuous estrous syndrome has been described. Also, the modifications observed in estrogen levels of neonatally melatonin-administered rats could explain the changes in estrous cyclicity reported for these animals.

From our study and from data of the literature we can conclude that depending upon dose, age and time of photophase in which melatonin is administered, the effects on the reproductive axis may differ. It seems that the modifications induced by prolactin and FSH on the peripubertal age could be responsible for the precocious puberty shown in this study.

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