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Prenatal melatonin exposure influences the maturation of gonadotropin and prolactin estradiol-benzoate feedback system

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Abstract

Gonadotropin and prolactin response to estrogen feedback in female rat offspring of control and melatonin treated (150 μg/100 g BW) mother rats during pregnancy (MEL-offspring) were studied at these periods: infantile, prepubertal and pubertal. In controls negative or absent LH feedback developed after estradiol benzoate (EB) injection up to 30 days of age indicating that the onset of puberty had not occurred. The positive feedback was established from day 33 on. However, in MEL-offspring the first activation of gonadotropin secretion during afternoon, 31 h after EB, was observed at 25 days of age, representing the first neuroendocrine sign of the onset of puberty. This positive response disappeared on day 30 in MEL-offspring. At 33 days of age, the LH positive response to EB was found in both groups, indicating a more advanced sexual development. In controls, this response increased at 35 days of age while in MEL-offspring it was highly depressed. FSH secretion in response to EB showed a negative feedback effect from infantile to the end of prepubertal period in both groups. The positive feedback was observed earlier in MEL-offspring (at 33 days of age) than in controls (at 35 days of age), but at this age it was absent in MEL-offspring. A positive prolactin response to EB at all ages in controls was observed. The typical pulsatility with higher values in the afternoon appeared by the first time at 30 days of age. However, in MEL-offspring no pulsatile response was observed throughout any age. These data suggest that prenatal melatonin administration altered gonadotropin and prolactin response to EB inducing precocious sensitivity during prepubertal period but depressed response during the pubertal period.

1. Introduction

From studies performed to analyze the changes in the feedback mechanisms of estradiol on gonadotropin secretion around the time of puberty in female rat, it was found that physiological levels of estradiol are markedly effective in inhibiting gonadotropin release in prepubertal as compared to postpubertal animals [1]. Similarly, other authors [2], found a negative LH feedback in response to estradiol benzoate (EB) during the infantile period. After 19 days of age, however, the animals showed a biphasic pattern, with a negative feedback in the first hours followed by a positive effect at 31 or 55 h after the EB injection. Basal LH secretion is already pulsatile before the first preovulatory surge [1]. Other authors [3] suggested that the desensitization of the hypothalamus to the inhibitory effect of gonadal steroids on gonadotropins in female rats is a protracted process that starts about 1 week before the vaginal opening and extends until the onset of puberty. On the other hand, serum prolactin levels are low in prepubertal rats and increase sharply at the time of vaginal opening [4]. These authors also found that estrogen is a potent stimulator of prolactin secretion in the immature as well as in the adult female rat. There is evidence about the existence of feedback mechanisms between prolactin and melatonin together with

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the fact that both hormones have an inhibitory role on the regulation of the hypothalamic-pituitary gonadal axis [5].

Melatonin (MEL), the main hormone of the pineal gland, passes from the mother to the fetus because MEL crosses every biological barrier [6,7]. Therefore, MEL can affect postnatal neuroendocrine-reproductive axis development. It has been demonstrated that melatonin can act directly on the fetus to influence postnatal somatic and hormonal development [8–10]. The stimulatory effect of the mother's pineal gland on androgen levels in the testes of prepubertal rats [11] occurs early in life [12,13].

There is evidence that the photoperiod under which the parents and developing young are exposed before birth affect the postnatal sexual development. It was found that testicular response of prepubertal Siberian hamsters to a photoperiod of intermediate duration (14 h) was influenced by the shorter or longer photoperiod to which the parents and developing fetuses were exposed before birth [14]. In addition, other authors [15] found that in juvenile male hamsters exposed to 14 L postnatally, endogenous MEL production and serum FSH concentrations are influenced by photoperiodic information received during fetal life. There are no references, however, about the possible influence of prenatal melatonin or prenatal photoperiod information transmission to the fetus on the gonadotropin-steroid feedback during postnatal life.

In the present study, we have examined in detail, throughout sexual development, the changes in sensitivity of the gonadotropin and prolactin response to estrogen feedback in female offspring of control and melatonin-treated mother rats.

2. Material and methods

2.1. Animals

Female Wistar rats from our colony and weighing 240–280 g at the beginning of the experiment were used. Animals were housed under 12-h light/dark cycles (lights on at 08.00 am), at a room temperature of approximately 23°C. Standard rat chow and water were available ad libitum. Mother rats were divided into two groups: control ($N = 48$) and melatonin-treated ($N = 30$), mating pairs were held in polypropylene cages, one male with two females. Possible pregnancy was monitored by the presence of vaginal spermatozoa.

2.2. Melatonin treatment

Considering previous findings [6] in which 20 μCi of ^{3}H-acetyl-melatonin was administrated to pregnant rats, and that each fetus contained slightly more than 0.1% (20 nCi) of the injection dose, 150 μg MEL/100 g body weight were used in the present study. MEL (Sigma Chemical Co.) was dissolved in a small volume of absolute ethanol and diluted in 0.9% NaCl to a dose of 150 μg/100 g body weight. Melatonin treatment was given at end of the light phase, and daily throughout gestation. Control mother rats received ethanol/saline alone.

2.3. Offspring studies

In order to obtain 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 female offspring we followed Ojeda's classification [16] concerning postnatal maturation: (a) infantile period, between 8 and 21 days, animals were studied at 15 (Control $n = 25$; MEL $n = 16$) and 18 days of age (control $n = 18$; MEL $n = 13$); (b) juvenile or prepubertal period, extends from 21 to 32 days of age; animals were studied at 21 (control $n = 13$; MEL $n = 19$), 23 (control $n = 13$; MEL $n = 17$), 25 (control $n = 14$; MEL $n = 16$) and 30 (control $n = 10$; MEL $n = 19$) days of age; (c) pubertal period, extends from day 32 to the day of vaginal opening, animals being examined 33 (control $n = 16$; MEL $n = 14$) and 35 (control $n = 13$; MEL $n = 14$) days of age. The animals were divided into two groups: offspring of control mother rats (control-offspring) and offspring of melatonin-treated mother rats (MEL-offspring). In all female rats, vaginal smears were taken at 30, 33 and 35 days of age in both offspring of vehicle and melatonin-treated mother rats to study the possible effect of the melatonin on the vaginal opening.

2.4. Estradiol benzoate test

All female rats were treated with estradiol benzoate (EB) at the mentioned ages. EB (Sigma Chemical Co.) was dissolved in 1 ml of polyethylene glycol and injected at a dose of 50 μg/rat, contained in 0.02 ml of the solution, EB was administrated by sc. injection. Blood samples were obtained by jugular venipuncture under ether anesthesia, afterwards animals recover rapidly. One ml of blood was taken each time from the same animal, only at 15 days of age, other pups of the same litter were used. Blood-loss was not supplemented, because the experiment was carried out within two days and animals recover normal activity between one extraction time and the next one. Basal samples were taken at 10 a.m. and blood samples 24, 31, 48 and 55 h after EB administration were also obtained. Samples were immediately centrifuged at 4°C

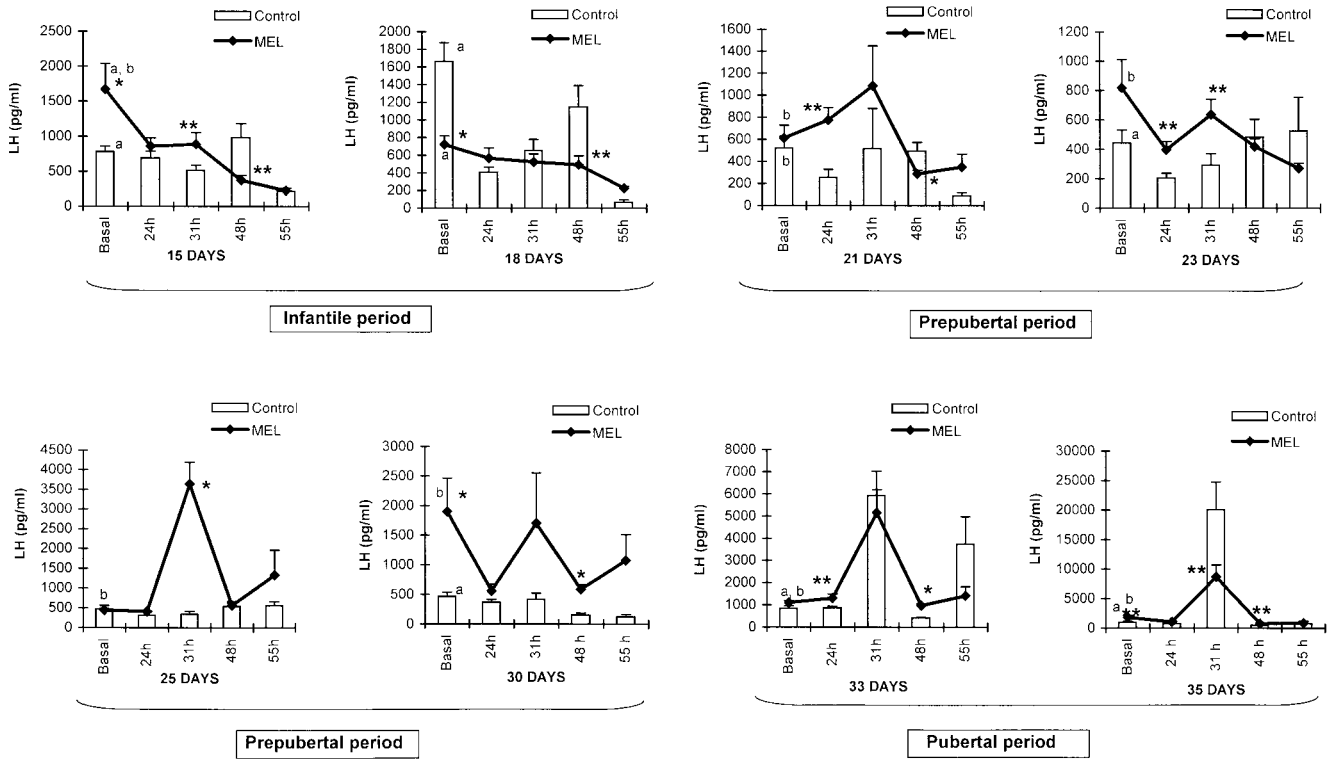

Fig. 1. Plasma LH response to estradiol benzoate (50 μg s.c.) administration on 15-, 18-, 21-, 23-, 25-, 30-, 33- and 35-day-old female offspring of control and melatonin treated (150 μg/100 g BW) mother rats. Control (n = 5–25); MEL (n = 9–19). Values are expressed as the mean ± S.E.M. *: $p < 0.01$; **: $p < 0.05$ vs. Control-offspring. Logitudinal study. Basal value vs. post-EB injection:15 days: control-offspring.(a) $p < 0.01$ vs. 55 h; MEL-offspring. (a) $p < 0.01$ vs. 55 h.; (b) $p < 0.05$ vs. 48 h. 18 days: control-offspring. (a) $p < 0.01$ vs. 24, 31 and 55 h; MEL-offspring. (a) $p < 0.01$ vs. 55 h. 21 days: control-offspring. (b) $p < 0.05$ vs. 55 h; MEL-offspring. (b) $p < 0.05$ vs. 48 and 55 h. 23 days: control-offspring. (a) $p < 0.01$ vs. 24 h.; MEL-offspring. (b) $p < 0.05$ vs. 24 and 55 h. 25 days: MEL-offspring. (b) $p < 0.05$ vs. 31 h. 30 days: control-offspring. (a) $p < 0.01$ vs. 48 and 55 h.; MEL-offspring. (b) $p < 0.05$ vs. 48 h. 33 days: control-offspring. (a) $p < 0.01$ vs. 31 and 48 h.; (b) $p < 0.05$ vs. 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 31 h. 35 days: control-offspring. (a) $p < 0.01$ vs. 31 h.; MEL-offspring. (b) $p < 0.05$ vs. 31 and 48 h.

and the plasma was separated and kept frozen at −20°C until analyzed.

2.5. Hormonal determinations

Plasma LH, FSH and prolactin levels were measured by specific double antibody-RIA systems employing materials kindly sent by the National Institute of Health (NIADDK, Bethesda, MD), and previously validated in our laboratory. Values of LH concentrations were expressed as pg/ml in terms of NIADDK rat LH-PR-3 (AFP, 71 87B). The sensitivity of the assay was 20 pg/ml. The final dilution of anti-rat LH-S-11 (AFP-C697071P) was 1:100.000 values of FSH were expressed in ng/ml of FSH-RP-2, the sensitivity of the assay being 95 pg/ml. The final dilution of anti-rat FSH-S-11 (AFP-CO 972 881) was 1:750.000. Values of prolactin were expressed in pg/ml of rat prolactin RP-3, the sensitivity of the assay being 40 pg/ml. The final dilution of anti-rat prolactin-S-9 was 1:5.000. All samples were measured in the same assay in order to avoid interassay variation.

2.6. Statistical analysis

Data of each age group were adjusted to a normal distribution test before being used in the statistical analysis. A 99 percentage of accuracy to normal distribution was required. Statistical analysis was performed using the SIGMA Statistical program (Copyright Horus Hardware, 1986). Results were expressed as mean ± S.E.M. At each age-studied comparison between groups at different time points studied were then made by one-way analysis of variance (ANOVA). A longitudinal study for each group between basal values against the rest of time points was performed by Student t-test. Mann–Whitney test for those cases with quasi significance value was used $P < 0.05$ was considered significant. Differences between both groups were noted by *: $p < 0.01$; **: $p < 0.05$. Differences in

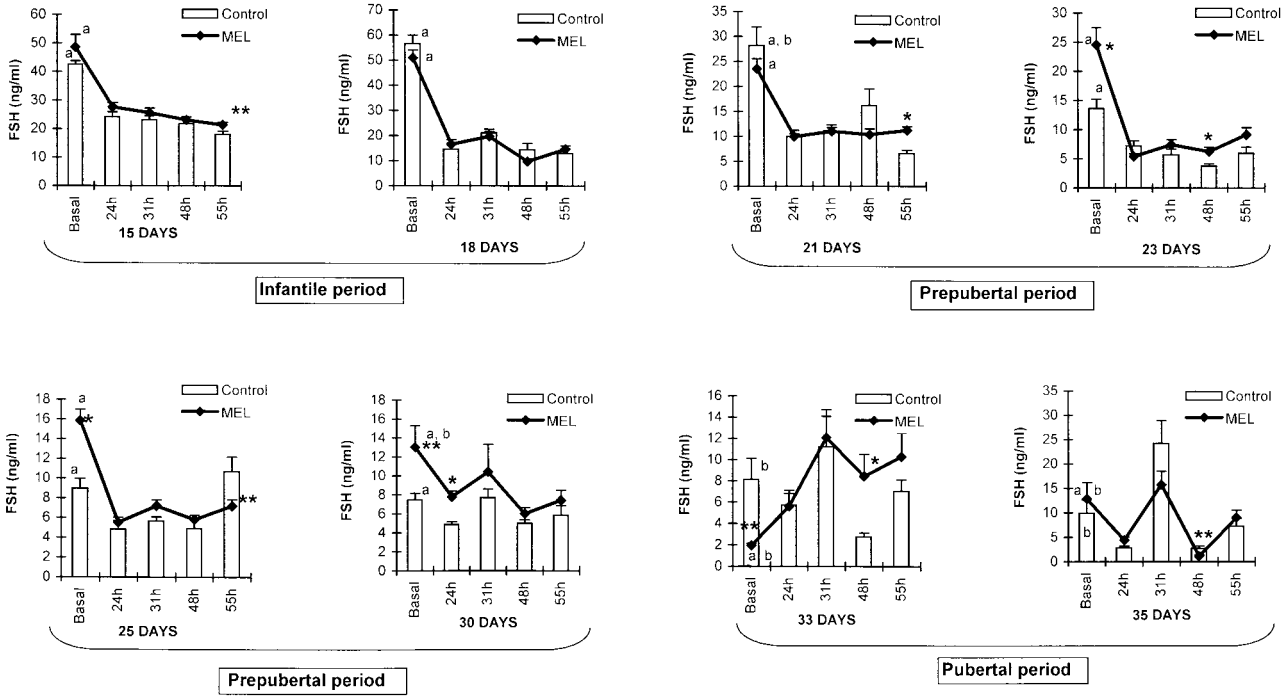

Fig. 2. Plasma FSH response to estradiol benzoate (50 μg s.c.) administration on 15-, 18-, 21-, 23-, 25-, 30-, 33- and 35-day-old female offspring of control and melatonin treated (150 μg/100 g BW) mother rats. Control (n = 6–24); MEL (n = 6–17). Values are expressed as the mean ± S.E.M. *: $p < 0.01$; **: $p < 0.05$ vs. control-offspring. Longitudinal study. Basal value vs. post-EB injection: 15 days: control-offspring. (a) $p < 0.01$ vs. 24, 31 and 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 18 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 21 days: control-offspring. (a) $p < 0.01$ vs. 24, 31 and 55 h.; (b) $p < 0.05$ vs. 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; 23 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 25 days: control-offspring. (a) $p < 0.01$ vs. 24, 31 and 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 30 days: control-offspring. (a) $p < 0.01$ vs. 24 and 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 48 h.; (b) $p < 0.05$ vs. 24 and 55 h. 33 days: Control-offspring. (b) $p < 0.05$ vs. 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 31 and 55 h.; (b) $p < 0.05$ vs. 24 and 48 h. 35 days: control-offspring. (b) $p < 0.05$ vs. 24 and 31 h.; MEL-offspring. (a) $p < 0.01$ vs. 48 h.; (b) $p < 0.05$ vs. 24 h.

feedback mechanisms, time-dependent were noted by: a: $p < 0.01$; b: $p < 0.05$.

3. Results

At 15 days of age basal LH values were significantly higher ($p < 0.01$) in MEL-offspring than in control-offspring (Fig. 1). Both groups showed a negative response to EB injection that was significant 55 h after EB injection, but in MEL-offspring it was found earlier at 48 h after EB injection, showing significantly lower values than in control-offspring. At 18 days of age basal LH values and 48 h after EB injection were significantly lower ($p < 0.01$; $p < 0.05$) in MEL-offspring than in control-offspring. At this age significantly decreased ($p < 0.01$) LH values in all time points studied except 48 h after EB injection were found in control-offspring. Whereas no decreased values were found in MEL-offspring until 55 h after EB ($p < 0.05$). At 21 days of age both groups showed significantly reduced ($p < 0.05$) LH values 55 h after EB injection. This negative response was observed earlier in MEL-offspring at 48 h showing at this time significantly lower ($p < 0.01$) values as compared to control-offspring. However 24 h after EB, MEL-offspring showed significantly increased ($p < 0.05$) LH values as compared to control-offspring. At 23 days of age EB administration to control-offspring significantly reduced ($p < 0.01$) LH values only 24 h after. However, MEL-offspring showed significantly reduced LH values ($p < 0.05$) 24 and 55 h after EB injection. At 25 days of age a lack of significant effects of EB injection on LH secretion was found in control-offspring. However, in MEL-offspring significantly increased ($p < 0.05$) values 31 h after EB injection were observed, at this time LH values were also significantly increased as compared to control-offspring. At 30 days of age basal LH values and 48 h after EB injection were significantly higher ($p < 0.01$) in MEL-offspring as compared to control-offspring. In control-offspring, significantly decreased ($p < 0.01$) LH values 48 and 55 h after EB injection were found and in MEL-offspring only 48 h after EB injection ($p < 0.05$). At 33 days of age in control-offspring a significant positive response ($p < 0.01$; $p < 0.05$) 31

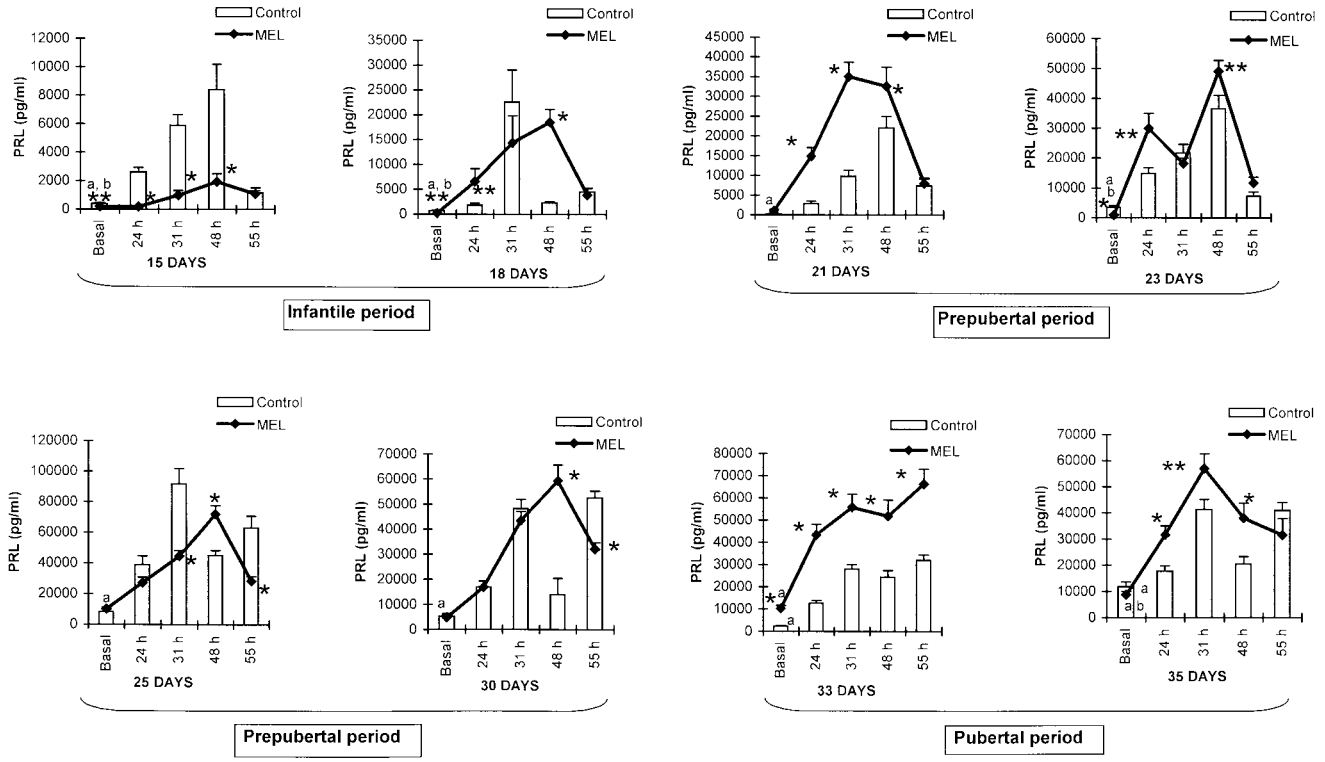

Fig. 3. Plasma prolactin response to estradiol benzoate (50 μg s.c.) administration on 15-, 18-, 21-, 23-, 25-, 30-, 33- and 35-day-old female offspring of control and melatonin treated (150 μg/100 g BW) mother rats. Control (n = 9–30); MEL (n = 9–21). Values are expressed as the mean ± S.E.M. *: $p < 0.01$; **: $p < 0.05$ vs. control-offspring. Longitudinal study. Basal value vs. post-EB injection:15 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 48 h.; (b) $p < 0.05$ vs. 31 and 55 h. 18 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 48 h.; (b) $p < 0.05$ vs. 31 and 55 h. 21 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 23 days: control-offspring. (a) $p < 0.01$ vs. 24, 31 and 48 h.; (b) $p < 0.05$ vs. 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 25 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 30 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 33 days: control-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h. 35 days: control-offspring. (a) $p < 0.01$ vs. 31 and 55 h.; (b) $p < 0.05$ vs. 24 and 48 h.; MEL-offspring. (a) $p < 0.01$ vs. 24, 31, 48 and 55 h.

and 55 h after EB injection was observed. In MEL-offspring the significant positive response ($p < 0.01$) was only found 31 h after EB injection. Differences between both groups studied were found, 24 and 48 h after EB injection, being LH values significantly higher ($p < 0.05$; $p < 0.01$) in MEL-offspring. At 35 days of age basal LH values and 48 h after EB injection were significantly higher ($p < 0.05$) in MEL-offspring as compared to control-offspring. 31 h after EB injection significantly increased ($p < 0.01$; $p < 0.05$) LH values were found in both groups, however in MEL-offspring were significantly lower ($p < 0.05$) than in control-offspring.

Plasma FSH values (Fig. 2) were similar in both groups studied at 15, 18, 21 and 23 days of age with significantly decreased ($p < 0.01$; $p < 0.05$) values after EB injection in all time points studied. At 25 days of age again EB injection resulted in significantly reduced FSH values ($p < 0.01$; $p < 0.05$) except 55 h after. At 30 days of age both groups showed significantly reduced FSH values 24 and 48 h after EB injections and after 55 h in MEL-offspring. However the negative response disappears after 31 h in both groups studied. Basal FSH levels were significantly higher ($p < 0.01$; $p < 0.05$) in MEL-offspring at 23, 25 and 30 days of age, but significantly lower at 33 days of age as compared to control-offspring. At 33 days of age, positive response to EB was observed in MEL-offspring in all time points studied, this response was not observed in control-offspring that showed negative response 48 h after EB injection. At 35 days of age, similar significant ($p < 0.05$) negative response was observed 24 h after EB injection in both groups studied this repeat 48 h after EB ($p < 0.01$) in MEL-offspring. However in control-offspring also a positive response was observed, with significantly increased ($p < 0.01$) FSH values 31 h after EB injection.

Basal prolactin values (Fig. 3) were significantly lower ($p < 0.05$) in MEL-offspring than in control-offspring during infantile period. Through the prepubertal period basal prolactin levels were similar in both groups studied except at 23 days of age, when signifi-

cantly reduced ($p < 0.01$) values were found in MEL-offspring. During pubertal period only at 33 days of age, significantly higher ($p < 0.01$) prolactin values in MEL-offspring as compared to control-offspring were observed. In control-offspring since infantile to pubertal period significantly increased prolactin values ($p < 0.01$; $p < 0.05$) in all time points after EB injection were found. Similar response was observed in MEL-offspring ($p < 0.01$) during prepubertal and pubertal periods. However at infantile period the positive response observed after EB injection was delayed until 31 h ($p < 0.01$; $p < 0.05$). Differences between both groups studied after EB injection were also observed. At 15 days of age, significantly reduced ($p < 0.01$) prolactin values in MEL-offspring as compared to control-offspring were observed, however at 18, 21 and 23 days of age significantly increased ($p < 0.01$; $p < 0.05$) prolactin values were observed as compared to controls. At 25 days of age, significantly increased ($p < 0.01$) prolactin values were observed in MEL-offspring 48 h after injection, but significantly reduced ($p < 0.01$) 31 and 55 h after EB injection. In a similar way, at 30 days of age, significantly higher ($p < 0.01$) prolactin values 48 h after EB, but significantly lower ($p < 0.01$) 55 h after were found. During pubertal period, MEL-offspring showed significantly increased ($p < 0.01$; $p < 0.05$) prolactin values as compared to control-offspring.

4. Discussion

The results of this investigation show that a negative or absent LH feedback effect developed after estradiol administration in control-offspring up to 30 days of age and the positive feedback was established from day 33 on. This is in agreement with previous reports that describe a different sensitivity of the gonadotropin releasing system of female rats to the inhibitory feedback effect of estradiol depending on the stage of the sexual development [1,17]. This response was altered in MEL-offspring, during the prepubertal phase, when, an increased LH secretion was found on day 25, 31 h after EB injection. Puberty is initiated at the point when levels of endogenous estrogen present in the system are no longer an effective inhibitor of LH and FSH secretion [18]. We can conclude that melatonin administration to the mothers induces precocious initiation of puberty at 25 days of age, when a single exogenous dose of 50 μg of EB was administered. However at 30 days of age the positive LH response was lost in MEL-offspring, showing at this age again a negative feedback effect 48 h after EB and in controls a negative feedback effect was found during the second postinjection day. This indicates that the onset of puberty did not occur, which was evidenced by the absence of vaginal opening in both groups. At 33 days of age, when 56.25–64.28% of animals in both groups studied showed vaginal opening, the LH positive feedback response 31 h after EB injection was observed, indicating a more advanced sexual development. And in control-offspring this positive response still increased at 35 days of age while in MEL-offspring it remained at similar levels to 33 days of age and was highly depressed showing a twofold lower increase as compared to control-offspring. Although the percentage of rats with vaginal opening increased at this age up to 69.23–71.42% in both groups studied. However, it is known that vaginal opening was not always preceded by a gonadotropin discharge, suggesting that the sequences of these events depends upon the relative threshold of vaginal tissue and of the gonadotropin-releasing system to estrogens stimulation [19]. The prevailing hypothesis regarding the initiation of puberty is based upon maturational shifts in hypothalamic sensitivity to and consequent feedback effects of estrogen, which may regulate gonadotropin release through feedback mechanisms [20]. Recent evidence indicates that the preovulatory surge of gonadotropins is induced, in part, by the disengagement of inhibitory synaptic connections in the hypothalamic arcuate nucleus [21]. Our data indicate that prenatal melatonin that may cross the placental barrier [6], can affect since intrauterine life the postnatal sexual maturation by altering the hypothalamic-pituitary axis response to ovarian estrogens as well as affecting basal values mainly during infantile period. Indeed the altered LH feedback to estrogens points to the hypothalamus as the putative level for prenatal melatonin influence. This is in agreement with most data that indicate that the major site of melatonin action is within the central nervous system [22,23].

Our results also indicate that the dose of melatonin used can affect the sexual development in different ways, because a delayed vaginal opening was found when higher melatonin doses were given prenatally [24,25]. This dose-dependent effect upon sexual development had also been previously described [26], although a lack of effect of different doses of melatonin was also reported in the rat [27].

FSH secretion in response to EB in control-offspring and MEL-offspring showed a clear negative feedback effect during the infantile period and also at the beginning of prepubertal phase. Alterations of FSH, negative feedback mechanisms to EB injection appear at 25 days of age in control-offspring but in MEL-offspring the clear negative feedback extended longer, until the end of prepubertal period (day 30 of life). This partially may be due to the higher basal FSH values present in this group during this period. Similarly to what was found in the LH response to EB, during the pubertal phase of the sexual development the positive feed-

back effect was observed earlier in MEL-offspring, at 33 days of age, while in the control-offspring it was apparent at 35 days of age. At this last age, in MEL-offspring this positive feedback response did not persist, similarly to what was found in LH response to EB. All this data point to the existence of differential sensitivity of the gonadotropin-releasing system to estrogen negative feedback from offspring of control and MEL-treated rats. The existence of the three major periods of activation of gonadotropin secretion during postnatal development has been suggested [28]. The first activation period occurs during infantile development as an enhancement in FSH secretion, with sporadic elevation in LH levels. The second activation period signals the end of juvenile development and represents the first neuroendocrine manifestation of the onset of puberty. Finally, the third activational period occurs more abruptly, and is predominantly determined by an increased output of ovarian steroids, especially estradiol. There is little doubt that the last phase represents the manifestation of an estradiol positive feedback. In relationship to this question, we observed that in the MEL-offspring, although the first neuroendocrine manifestation of the onset of puberty occurs earlier at 33 days of age, then it was absent and the third activation period did not occur as abruptly as was observed in control-offspring. This is in agreement with previous results that point to a melatonin influence on the sexual maturation of the rat, via the hypothalamus, by interfering with GnRH release and having an hormonal pattern of low frequency of proestrus surges in MEL-treated rats [29].

It is known that estrogens are potent stimulators of prolactin secretion [30], in intact immature and adult female rats [4]. From our results, a prolactin positive response to EB at all ages studied of control-offspring and MEL-offspring was observed. The typical pulsatility with higher values in the afternoon than in the morning after EB injection previously observed in adult rats [2], appeared in our study by the first time in control-offspring during the prepubertal phase, at 30 days of age. A different pattern of development was observed in MEL-offspring. No pulsatile response was observed throughout all ages studied. However it is clear from the experiments here presented that prenatal melatonin has a stimulatory action on prolactin release in response to EB at all ages studied. Only at the infantile period where the feedback effects between estradiol and prolactin were slower developed in MEL-offspring than in control-offspring, also basal prolactin secretion pattern in MEL-offspring showed a delayed increase in this period of the sexual development. All these data suggest that the effect of prenatal melatonin modulate prolactin secretion in response to EB from the infantile to the pubertal period in female offspring. An interrelationship between melatonin and prolactin has already been described in the rat [31,32]. On the other hand, estrogens exert a dual action upon prolactin secretion in control rats, one of them is a direct effect on the pituitary gland, and another potent through the hypothalamus indicated by the strong pulsatile response [33]. Having in mind that specific hypothalamic melatonin receptors have been previously described [23]; our results with absent pulsatility in MEL-offspring suggest a direct action of maternal melatonin at hypothalamic level.

All these data suggest that prenatal melatonin treatment affect the postnatal development of gonadotropin and prolactin feedback mechanisms to EB as early as in intrauterine life.

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