PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 72 (2000) 61–69

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Effect of prenatal melatonin on the gonadotropin and prolactin response to the feedback effect of testosterone in male offspring

Elena Díaz[a], Patricia Castrillón[b], Ana Esquifino[b], Beatriz Díaz[a,*]

[a]*Dpto. Biología Funcional, Area Fisiología, Fac. Medicina, Universidad de Oviedo, Oviedo, Spain*
[b]*Dpto. de Bioquímica y Biología Molecular III, Fac. Medicina, Universidad Complutense, Madrid, Spain*

Received 23 June 1999; accepted 13 August 1999

Abstract

The purpose of this study was to investigate the effects of prenatal melatonin administration on the sensitivity of the androgens negative feedback effect on gonadotropin and prolactin secretion in male offspring. Male offspring of control (control-offspring) and melatonin treated (MEL-treated) (150 μg/100 g BW) mother rats during pregnancy (MEL-offspring), at infantile, prepubertal, and pubertal periods were studied. LH secretion in response to testosterone propionate (TP) in control-offspring showed the classical negative feedback effect at all ages studied. In MEL-offspring a negative response after TP was also observed in all ages studied although the magnitude of this response was altered in this group as compared to controls. FSH values were significantly lower at most ages and time points studied in MEL-offspring than in control-offspring. FSH secretion in MEL-offspring showed a delayed negative feedback action of TP injection as compared to control-offspring. This response was observed at 21 days of age in control-offspring and delayed until day 30 of life in MEL-offspring. Parallely it remain at later age in MEL-offspring than in control-offspring. Prolactin secretion in control-offspring showed increased values after TP injections from infantile to pubertal periods. This increase was blunted in MEL-offspring at 17 and 35 days of age showing significantly reduced ($p < 0.01$; $p < 0.05$) plasma prolactin levels. During pubertal period a prolactin positive response to TP administration was observed in MEL-offspring but with significantly lower magnitude than in control-offspring. These results indicate that prenatal melatonin exposure induced changes in the sensitivity of gonadotropin and prolactin feedback response to testosterone, indicating a delayed sexual maturation of the neuroendocrine-reproductive axis in male offspring.

1. Introduction

It is well established that the interaction of testicular androgens with the hypothalamus shortly after birth in male rats is the mechanism that leads to the tonic type of hypothalamic control of gonadotropin secretion [1]. Gonadotropin secretion in prepubertal male rats has been shown to be maintained at low levels by a central restraining mechanisms extremely sensitive to gonadal steroid feedback [2,3]. At puberty sensitivity to gonadal steroid negative feedback declines sharply [3]. The modification in the sensitivity of the negative feedback effect of gonadal steroids on gonadotropin secretion is one of the principal events involved in the onset of puberty [4]. Much less testosterone is needed to suppress LH levels in prepubertal than in pubertal or adult males which reflect a "shift" at puberty in the response of gonadotropins to androgens. At normal puberty in male rats there is no clear change in plasma LH levels [5]. On the other hand, changes in prolactin levels are able to modify the sensitivity of the negative feedback effect of gonadal steroids on gonadotropin secretion [6], and influence the onset of puberty in female rats [7]. The different effects of prolactin on hypothalamic structures depend on the developmental status of these structures [8]. The serotoninergic system involved in prolactin release is stimulated by testoster-

* Corresponding author. Tel.: +34-98-510-27-13; fax: +34-98-510-35-34.
E-mail address: beatrizd@correo.uniovi.es (B. Díaz).

0960-0760/00/$ - see front matter
PII: S0960-0760(99)00150-8

one during the prepubertal stage, but not in peripubertal or adult rats. This could be a regulatory mechanism operating during the pubertal stage, which modifies the sensitivity of the gonadostat to the negative feedback effect of testosterone and thus, could be related to the different physiological process involved in the onset of puberty. This suggests an additional participation of testosterone in the neuroendocrine mechanisms involved in sexual maturation [9].

Pineal gland may exert a modulatory action in the onset of puberty through changes in melatonin production [10,11]. Melatonin may cross placental barrier [12], therefore, maternal melatonin may affect the postnatal sexual and somatic development of the offspring. We previously found that melatonin treatment to mother rats can act on fetal development and influence the postnatal ontogeny of the hormones involved in the neuroendocrine-reproductive axis in developing rats [13]. In addition, it was demonstrated [14] that exogenous melatonin given to pregnant female Siberian hamsters at particular times of the day affects the postnatal testicular development of the prepubertal male offspring. In this way, it was demonstrated that the photoperiodic information received prior to birth influence testicular development between days 15 and 28 in Syrian hamsters kept under constant light [15]. From these data, it is assumed that maternal melatonin mediate the effects of prenatal photoperiods on development of fetal neuroendocrine system regulating postnatal reproductive development [14].

The purpose of this investigation was to examine the role of prenatal melatonin administration on the shift in the steroid feedback process of gonadotropin and prolactin response throughout sexual development of male offspring.

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 as mother rats. 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 tap 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

Based on previous findings [12] 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, the dose of melatonin chose in the present investigation was 150 μg MEL/100 g BW. MEL (Sigma) was dissolved in a small volume of absolute ethanol and then diluted in 0.9% NaCl. Melatonin injections were given sc. at the 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 male offspring of control mother rats (Control-offspring) and of MEL-treated mother rats (MEL-offspring), we followed the classification proposed by Ojeda [16] concerning postnatal maturation: (a) Infantile period, between 8 and 21 days, animals were studied at 17 (Control $n = 15$; MEL $n = 14$) and 21 days of age (Control $n = 16$; MEL $n = 16$); (b) Juvenile or prepubertal period, extends from 21 to day 35; animals were studied at 30 (Control $n = 17$; MEL $n = 18$), and 35 (Control $n = 15$; MEL $n = 18$) days of age; (c) Pubertal period, extends from day 35 to days 55–60, animals being examined at 40 (Control $n = 16$; MEL $n = 17$) and 60 (Control $n = 9$; MEL $n = 12$) days of age.

2.4. Testosterone propionate test

All male offspring received a single dose of testosterone propionate (TP) at the mentioned ages. TP (Sigma) was dissolved in polyethylene glycol and injected at a dose of 100 μg/100 g BW, by s.c. injection contained in approximately 0.2 ml of the solution. Blood samples were obtained by jugular venipuncture under slight ether anesthesia. Afterwards animals recovered rapidly, 1 ml of blood was taken each time point from the same animal. Blood lost was not supplemented, because the extractions were carried out with sufficient time interval to allows the animal's recuperation. Basal samples were taken at 10 am and blood samples 8, and 24 h after TP administration were also obtained. Samples were immediately centrifuged at 4°C 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 furnished by the National Institute of

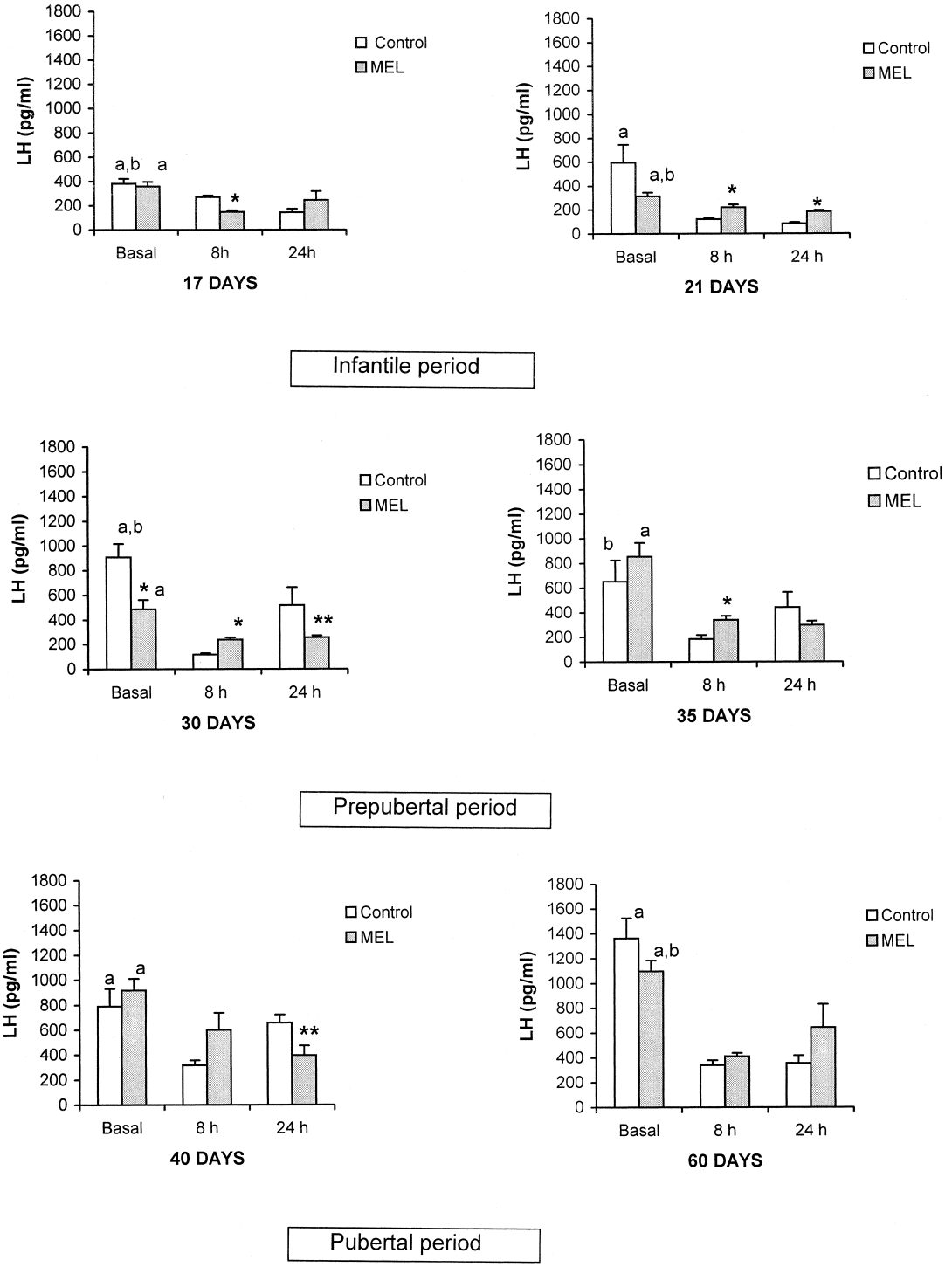

Fig. 1. Plasma LH response to testosterone propionate (100 μg/100 g BW) administration on 17-, 21-, 30-, 35-, 40-, and 60-day old male offspring of control and melatonin-treated (150 μg/100 g BW) mother rats. Control ($n = 8$–15); MEL ($n = 5$–18). Values are expressed as the mean ± SEM. $*p < 0.01$ vs. Control group; $**p < 0.05$ vs. Control group. Longitudinal study: Basal value vs. post TP injection: 17 days, Control-offspring: (a) $p < 0.01$ vs. 24 h, (b) $p < 0.05$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 8 h. 21 days, Control-offspring: (a) $p < 0.01$ vs. 8 and 24 h; MEL-offspring: (a) $p < 0.01$ vs. 24 h, (b) $p < 0.05$ vs. 8 h. 30 days, Control-offspring: (a) $p < 0.01$ vs. 8 h, (b) $p < 0.05$ vs. 24 h; MEL-offspring: (a) $p < 0.01$ vs. 8 and 24 h. 35 days, Control-offspring: (b) $p < 0.05$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 8 and 24 h. 40 days, Control-offspring: (a) $p < 0.01$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 24 h. 60 days, (a) $p < 0.01$ vs. 8 and 24 h; MEL-offspring: (a) $p < 0.01$ vs. 8 h, (b) $p < 0.05$ vs. 24 h.

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 1:75,000. 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 1:5000. All samples were measured in the same assay in order to avoid interassay variation.

2.6. Statistical analysis

Statistical analysis was performed using the SIGMA Statistical program (Copyright Horus Hardware, 1986). Results were expressed as mean ± SEM. Comparisons between both groups of all data of gonadotropins and prolactin concentrations at all ages studied were determined by one-way analysis of variance (ANOVA). Mann–Whitney test for those cases with borderline significance value was used. Direct comparison between basal and post-TP injection was made in each group by Student's T-test. Differences between both groups were noted by $*$: $p < 0.01$; $p < 0.05$. Differences in feedback mechanisms, time-dependent were noted by (a) $p < 0.01$ and (b) $p < 0.05$.

3. Results

Basal LH values and feedback mechanism to PT (Fig. 1). From 17 to 60 days of age basal LH values were only affected by maternal melatonin at 30 days of age, showing significantly reduced ($p < 0.01$) values as compared to control-offspring.

3.1. LH feedback mechanism

At 17 days of age, in control-offspring significantly reduced ($p < 0.05$; $p < 0.01$) LH values were found 8 and 24 h after TP injection. In MEL-offspring this response was only observed 8 h after showing significantly lower ($p < 0.01$) values as compared to control-offspring. At 21 days of age, significantly decreased ($p < 0.01$; $p < 0.05$) LH values after TP injection were found in both groups studied but in MEL-offspring LH values were significantly higher than ($p < 0.01$) in control-offspring, in both time points studied. At 30 days of age, again significantly reduced ($p < 0.01$; $p < 0.05$) LH values after TP injection were found in both groups studied. But the magnitude of the negative response was significantly higher ($p < 0.01$) 8 h after in control-offspring and 24 h after in MEL-offspring ($p < 0.05$). At 35 days of age, significantly reduced ($p < 0.05$; $p < 0.01$) LH values 8 h after TP injection were found in both groups, showing significantly higher ($p < 0.01$) LH values in MEL-offspring as compared to control-offspring. This negative response ($p < 0.01$) was only observed in MEL-offspring 24 h after. At 40 days of age, significantly decreased ($p < 0.01$) LH values 8 h after TP injection were found in control-offspring, but this response was delayed ($p < 0.01$) in MEL-offspring until 24 h after, being at this time LH values significantly lower ($p < 0.05$) as compared to controls. At 60 days of age, both groups showed significantly decreased ($p < 0.01$; $p < 0.05$) LH values 8 and 24 h after TP injection.

Basal FSH values and feedback mechanism to TP (Fig. 2). Basal FSH values were significantly lower at all ages studied except at 60 days of age in MEL-offspring as compared to control-offspring.

3.2. FSH feedback mechanism

In control-offspring at 17 days of age, no FSH response to a single dose of TP was found. At the end of the infantile period (21 days), FSH values were significantly reduced ($p < 0.05$) 24 after TP injection. During prepubertal period (days 30 and 35) significantly reduced FSH values ($p < 0.01$; $p < 0.05$) were found 8 and 24 h after TP. At pubertal period (days 40 and 60), no negative response was found after TP injection. However, in MEL-offspring the significantly negative ($p < 0.01$; $p < 0.05$) FSH response appeared later than in control-offspring during prepubertal and disappeared delayed than in control-offspring at the beginning of the pubertal period. At all ages studied, significantly lower ($p < 0.01$; $p < 0.05$) FSH values were found after TP injection as compared to control-offspring.

Basal prolactin values and feedback mechanism to TP (Fig. 3). Basal prolactin levels were significantly lower ($p < 0.01$) in MEL-offspring during the infantile period and at 35 days of age than in control-offspring.

3.3. Prolactin feedback mechanism

In control-offspring, significantly increased ($p < 0.01$) prolactin levels were observed 8 h after TP injection at all ages studied except at 17 days of age. At this age, significantly negative response ($p < 0.05$) was observed 8 h after TP injection, but 24 h afterward prolactin values increased showing significantly higher ($p < 0.01$) values than in MEL-offspring. The increased prolactin levels observed in control-offspring after TP injection were blunted in MEL-offspring at 17 and 35 days of age, showing significantly reduced ($p < 0.01$; $p < 0.05$) prolactin levels than control-off-

spring. Also, at 21 days of age, prolactin levels were significantly lower ($p < 0.01$) in all time points studied in MEL-offspring than in control-offspring. Although at this age prolactin positive response to TP was found 8 and 24 h after in MEL-offspring. On day 30 of life, no significant differences were found between the two groups studied. In MEL-offspring during the pubertal period, 40 and 60 days of age, increased prolactin

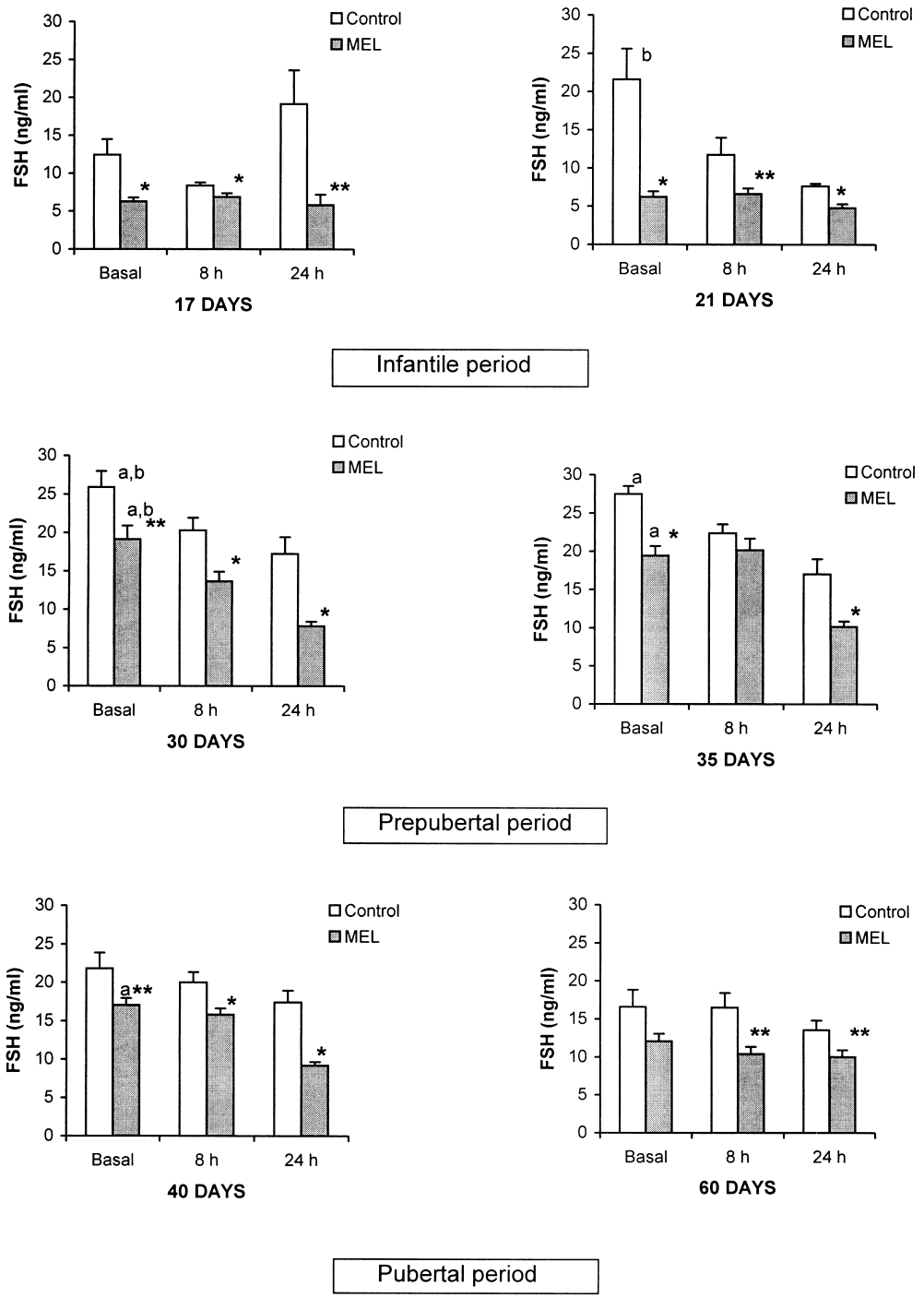

Fig. 2. Plasma FSH response to testosterone propionate (100 μg/100 g BW) administration on 17-, 21-, 30-, 35-, 40-, and 60-day old male offspring of control and melatonin-treated (150 μg/100 g BW) mother rats. Control ($n = 7$–16); MEL ($n = 7$–17). Values are expressed as the mean ± SEM. $*p < 0.01$ vs. Control group; $**p < 0.05$ vs. Control group. Longitudinal study: Basal value vs. post TP injection: 21 days, Control-offspring: (b) $p < 0.05$ vs. 24 h. 30 days, Control-offspring: (a) $p < 0.01$ vs. 24 h, (b) $p < 0.05$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 24 h, (b) $p < 0.05$ vs. 8 h. 35 days, Control-offspring: (a) $p < 0.01$ vs. 8 and 24 h; MEL-offspring: (a) $p < 0.01$ vs. 24 h. 40 days, MEL-offspring: (a) $p < 0.01$ vs. 48 h.

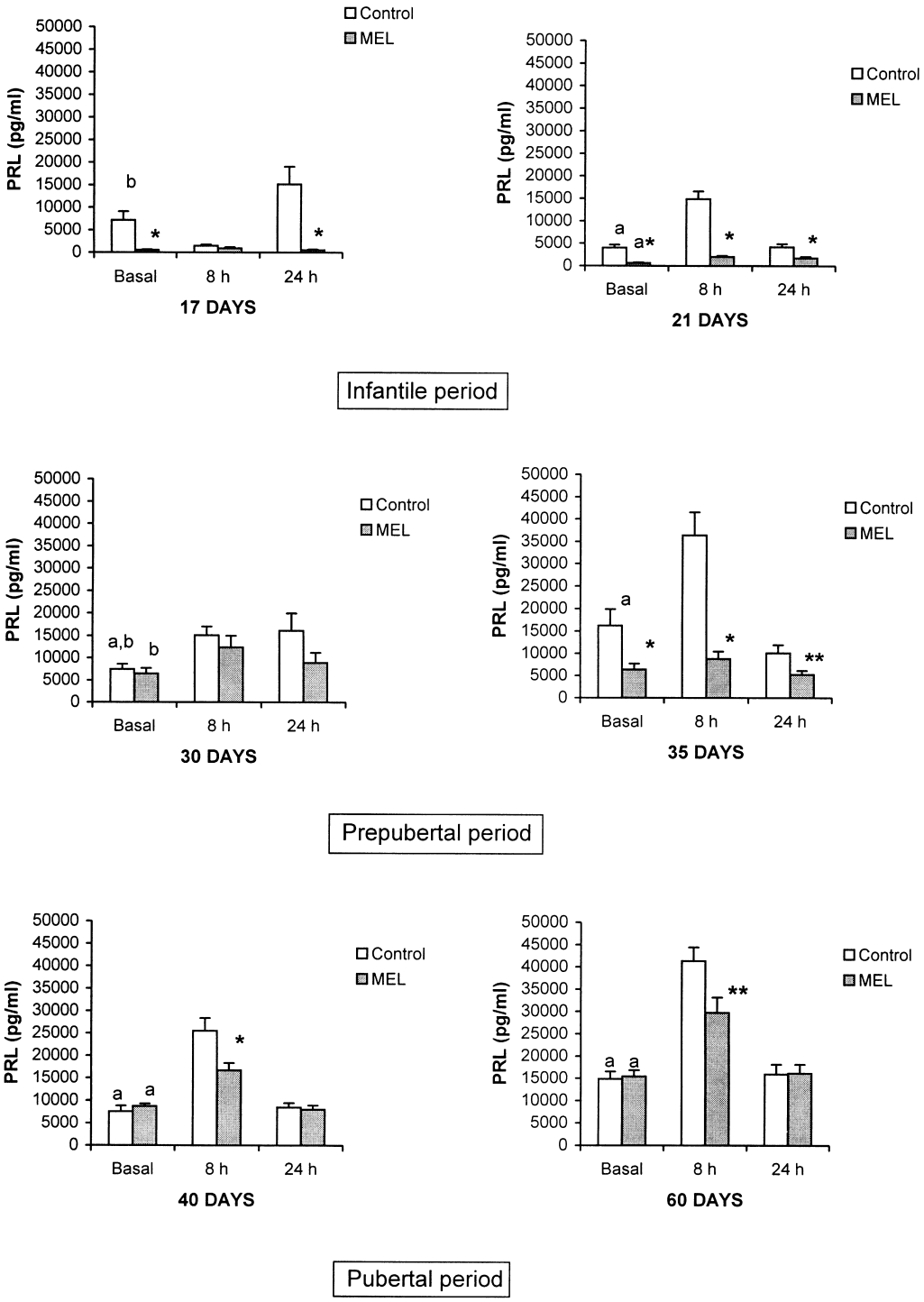

Fig. 3. Plasma prolactin (PRL) response to testosterone propionate (100 μg/100 g BW) administration on 17-, 21-, 30-, 35-, 40-, and 60-day old male offspring of control and melatonin-treated (150 μg/100 g BW) mother rats. Control ($n = 9$–16); MEL ($n = 8$–18). Values are expressed as the mean ± SEM. $*p < 0.01$ vs. Control group; $**p < 0.05$ vs. Control group. Longitudinal study: Basal value vs. post TP injection: 17 days, Control-offspring: (b) $p < 0.05$ vs. 8 h. 21 days, Control-offspring: (a) $p < 0.01$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 8 and 24 h. 30 days, Control-offspring: (a) $p < 0.01$ vs. 8 h, (b) $p < 0.05$ vs. 24 h; MEL-offspring: (b) $p < 0.05$ vs. 8 h. 35 days, Control-offspring: (a) $p < 0.01$ vs. 8 h. 40 days, Control-offspring: (a) $p < 0.01$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 8 h. 60 days, Control-offspring: (a) $p < 0.01$ vs. 8 h; MEL-offspring: (a) $p < 0.01$ vs. 8 h.

values were found 8 h after TP injection but this stimulatory effect was significantly lower ($p < 0.01$; $p < 0.05$) than in control-offspring.

4. Discussion

The present study clearly demonstrates that modifications of the fetal endocrine environment caused by prenatal administration of melatonin resulted in an alteration of the neuroendocrine-reproductive axis of the male offspring.

In control-offspring, LH levels in response to TP, showed the classical negative feedback effect as early as 8 h after TP injection, since infantile period up to pubertal period. The highest magnitude of the negative response from infantile to pubertal period was observed at 30 days of age. About this question, at 30 days of age increased pituitary GnRH receptors were described in male rats [17]. However, using the same TP doses (100 μg/100 g BW), 5 days later the negative response was attenuated. This indicate that at 35 days of age, which is a transitional stage from the prepubertal to the pubertal period, the neural mechanisms involved in feedback response are at a more advanced phase of sexual development. Our results are in agreement with previous data in the literature, which reported that much less testosterone is needed to suppress LH levels in prepubertal than in pubertal males [5] and that a functional sensitive androgen feedback system exists in immature male rats [18]. About this matter increased serum gonadotropins in 5–60 days of age castrated male rats were found [19], suggesting that the negative feedback action of gonadal steroids on hypothalamic GnRH secretion is operative since early age. In MEL-offspring where similarly the negative response to TP was observed as early as 17 days of age. The highest magnitude of the negative response to TP was observed 5 days later than in control-offspring, at 35 days of age, as consequence of delayed increase of basal LH values. Recently, it was demonstrated that androgen feedback action is exerted at hypothalamic level because testosterone treatment had no effect on LH secretion in GnRH-pulsed castrated male rams which underwent hypothalamo-pituitary disconnection [20]. These data suggest that prenatal melatonin acts upon the hypothalamic-pituitary axis producing alterations in the sensitivity to gonadal steroids during the prepubertal period, supported by the evidence that melatonin acts at the level of the hypothalamus or on higher centers to inhibit GnRH release and reproductive functions [10,21–23].

The results show a different developmental pattern of the two gonadotropins in response to testosterone through sexual development in control-offspring. These observations of TP differential effects on FSH and LH secretion suggest that their secretion may be controlled by different mechanism, as was previously proposed in rats [24,25]. FSH secretion showed a more reduced negative feedback response to supraphysiological androgen levels in immature rats than the one observed with LH. A more delayed negative feedback of testosterone on serum FSH as compared with LH was recently described [26]. Which could be due to that additional factors other than gonadal steroids are operative in males during maturation. It was recently found [27,28] that FSH secretion in addition to testosterone is regulated by other factors, such as inhibin B, which plays a clear physiological role in the feedback control. In MEL-offspring decreased FSH concentrations in most time points studied and delayed negative feedback effect were observed. About this sensitive mechanism in MEL-offspring the negative feedback was observed by first time at 30 days of age while in control-offspring it was observed at 21 days of age. This negative feedback effect to exogenous TP disappear at 35 days of age in control-offspring while in MEL-offspring it was delayed until 40 days of age. Maturation processes of pituitary responsiveness to both GnRH and steroid feedback action are probably the most important steps in the regulation of sexual maturation in the rat. All these data suggest that prenatal melatonin induced a delay in the sexual development of male offspring. This effect could be exerted at hypothalamic level since it is known that the effects of melatonin on the decreased function of gonadotrophs of the male rat result from an action exerted at hypothalamic rather than at the pituitary level [29]. All these data suggest that the hypothalamus is the site where prenatal melatonin exerts its influence and alters the postnatal FSH response to androgens in male offspring.

The results from the present study show that plasma prolactin levels increase from infantile to pubertal periods, in agreement with previous results [30]. As was previously found [9], TP produced a stimulatory action on the mechanisms involved in prolactin release in control-offspring at all ages studied. In MEL-offspring the increased prolactin values after TP injection were blunted at 17 and 35 days of age. Moreover, prenatal melatonin altered the prolactin response to exogenous TP, producing during prepubertal period an inhibition of the positive feedback observed at 35 days of age in control-offspring. This could be as a consequence that at this age in control-offspring basal prolactin levels markedly increase; however, in MEL-offspring they remain at the same level observed at 30 days of age. During the pubertal period, although MEL-offspring group showed a positive feedback, the magnitude of the response was lower than in control-offspring. This coincide with the results observed in FSH response at the same period. Based on these

data, we concluded that prenatal melatonin could act upon central structures to affect postnatal prolactin-secretion in response to TP injection until puberty.

In conclusion, the present study shows that prenatal melatonin administration induced changes in the sensitivity of gonadotropin and prolactin feedback response to exogenous androgens, indicative of a delayed sexual maturation in male offspring.

Acknowledgements

This work was supported through a grant from the Spanish Ministry of Education and Science, DGCYT, PM 91-0225. The authors wish to express their gratitude to the NIADDK, NHPP and the University of Maryland School Medicine for the provisions of radioimmunoassay materials of LH, FSH and prolactin, and to Dr. Debeljuk for his grammatical assistance in preparing the manuscript.

References

[1] R.A. Gorski, Gonadal hormones and the perinatal development of neuroendocrine function, in: L. Martini, W.F. Ganong (Eds.), Frontiers in Neuroendocrinology, vol. II, Oxford University Press, New York, 1971, pp. 237–263.

[2] W.W. Byrnes, R.K. Meyer, Inhibition of gonadotrophic hormones secretion by physiological doses of estrogen, Endocrinology 48 (1951) 133–136.

[3] V.D. Ramirez, S.M. McCann, A comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats, Endocrinology 72 (1963) 452–464.

[4] A.M. Matsumoto, A.E. Karpas, M.B. Southworth, Evidence for the activation of the central nervous system-pituitary mechanisms for gonadotropin secretion at the time of puberty in male rats, Endocrinology 119 (1986) 362–369.

[5] G.J. Bloch, J. Masken, C.L. Kragt, W.F. Ganong, Effect of testosterone on plasma LH in male rats of various ages, Endocrinology 94 (1974) 947–951.

[6] A.S. McNeilly, R.M. Sharpe, H.M. Fraser, Increased sensitivity to the negative feedback effects of testosterone induced by hyperprolactinemia in the adult male rat, Endocrinology 112 (1983) 22–28.

[7] J.P. Advis, S.R. Ojeda, Hyperprolactinemia induced precocious puberty in the female rat: ovarian site of action, Endocrinology 103 (1978) 924–931.

[8] D. Cocchi, F. Fraschini, H. Jalanbo, E.E. Müler, Role of brain catecholamines in the postcastration rise in plasma LH of prepubertal rats, Endocrinology 95 (1974) 1649–1657.

[9] J.A. Moguilevsky, G. Justo, S. Justo, B. Szwarcfarb, S. Carbone, P. Scacchi, Modulatory effect of testosterone on the serotoninergic control of prolactin secretion in prepubertal rats, Neuroendocrinology 51 (1990) 197–201.

[10] R.J. Reiter, The pineal and its hormones in the control of reproduction in mammals, Endocinol. Rev. 1 (1990) 109–131.

[11] D.P. Cardinali, Melatonin, a mammalian pineal hormone, Endocrine Rev. 2 (1981) 327–331.

[12] D.C. Klein, Evidence for the placental transfer of ^{3}H-acetylmelatonin, Nature 237 (1972) 117–118.

[13] B. Díaz López, M.D. Colmenero Urquijo, M.E. Díaz Rodríguez, A. Arce Fraguas, A. Esquifino Parras, B. Marín Fernández, Effect of pinealectomy and melatonin treatment during pregnancy on the sexual development of the female and male rat offspring, European J. Endocrinol. 132 (1995) 765–770.

[14] T.H. Horton, S.L. Ray, S.M. Stetson, Maternal transfer of photoperiodic information in Siberian hamsters. Part III: Melatonin injections program postnatal reproductive development expressed in constant light, Biol. Reprod. 41 (1989) 34–39.

[15] M.H. Stetson, S.L. Ray, N. Creyaufmiller, T.H. Horton, Maternal transfer of photoperiodic information in Siberian hamsters. Part II: The nature of the maternal signal on peripubertal reproductive development in the absence of photoperiodic input, Biol. Reprod. 40 (1989) 458–465.

[16] S.R. Ojeda, W.S. Andrews, J.P. Advis, S. Smith-White, Recent advances in endocrinology of puberty, Endocrinol. Rev. 1 (1980) 228–257.

[17] D. Becu-Villalobos, A. González, G. Díaz-Torga, P. Hockl, C. Libertum, Brain sexual differentiation and gonadotropins secretion in the rat, Cell. Moll. Neurobiol. 17 (1997) 699–715.

[18] S.R. Ojeda, V.D. Ramirez, Plasma levels of LH and FSH in maturing rats: response to hemigonadectomy, Endocrinology 90 (1972) 466–472.

[19] J.A. Duncan, A.C. Dalkin, A. Barkan, S. Regiani, J.C. Marshall, Gonadal regulation of pituitary gonadotropin-releasing hormone receptors during sexual maturation in the rat, Endocrinology 113 (1983) 2238–2246.

[20] A.I. Tilbrook, D.M. de Kretser, I.J. Clarke, Seasonal changes in the negative feedback regulation of the secretion of the gonadotropins by testosterone and inhibin in rams, J. Endocrinol. 160 (1999) 155–167.

[21] J.D. Glass, G.R. Lynch, Evidence for a brain site of melatonin action in the white-footed mouse *Peromyscus leucopus*, Neuroendocrinology 34 (1982) 1–6.

[22] U. Lang, M.L. Aubert, B.S. Konne, J.C. Bradtke, P.C. Sizonenko, Influence of exogenous melatonin on melatonin secretion and on the neuroendocrine reproductive axis of intact male rats during sexual maturation, Endocrinology 112 (1983) 1578–1584.

[23] R.W. Rivest, U. Lang, M.L. Aubert, P.C. Sizonenko, Puberty in the rat: modulation by melatonin and light, J. Neural Transm. 21 (1986) 81–108.

[24] R.R. Grady, L. Shin, C.M. Charlesworth, I.R. Cohen-Becker, M. Smith, C. Rivier, J. Rivier, W. Vale, N.B. Schwartz, Differential supression of follicle-stimulating hormone and luteinizing hormone secretion in vivo by gonadotropin-releasing hormone antagonist, Neuroendocrinology 40 (1985) 246–252.

[25] M.D. Culler, A. Negro-Vilar, Evidence that pulsatile follicle stimulating hormone secretion is independent of endogenous luteinizing hormone-releasing hormone, Endocrinology 118 (1986) 609–612.

[26] C. Foresta, P. Bordon, M. Rossato, R. Mioni, J.D. Veldhuis, Specific linkages among luteinizing hormone, follicle stimulating hormone, and testosterone release in the peripheral blood and human spermatic vein: evidence for both positive (feed-forward) and negative (feedback) within-axis regulation, J. Clin. Endocrinol. Metab. 82 (1997) 3040–3046.

[27] R.A. Anderson, E.M. Wallace, N.P. Groome, A.J. Bellis, F.C.W. Wu, Physiological relationship between inhibin B, follicle stimulating hormone secretion and spermatogenesis in normal men and response to gonadotrophin suppression by exogenous testosterone, Human Reprod. 12 (1997) 746–751.

[28] A.J. Tilbrook, D.M. de Kretser, I.J. Clarke, Changes in the suppressive effects of recombinant inhibin A on FSH secretion in ram lambs during sexual maturation: evidence for alterations

in the clearance rate of inhibin, J. Endocrinol. 161 (1999) 219–229.

[29] M.L. Aubert, R.W. Rivest, U. Lang, B.P. Winiger, P.C. Sizonenko, Delayed sexual maturation induced by daily melatonin administration eliminates the LH response to naloxone despite normal responsiveness to GnRH in juvenile male rats, Neuroendocrinology 48 (1988) 72–80.

[30] K.D. Dohler, W. Wuttke, Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats, Endocrinology 97 (1975) 898–908.