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In vitro pituitary prolactin, growth hormone and follicle stimulating hormone secretion during sexual maturation of female rats primed with melatonin

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Abstract. The influence of *in vivo* melatonin administration on *in vitro* pituitary follicle stimulating hormone (FSH), growth hormone (GH) and prolactin secretion, as well as the possible influence of dopamine (DA) were evaluated in prepubertal (31-day-old), pubertal (33-day-old) and adult female rats at diestrus phase of the sexual cycle. The *in vitro* pituitary hormone secretions were evaluated at basal rate for the first hour of incubation only, in Krebs Ringer phosphate (KRP) (I_1) and after a second hour of incubation with KRP (I_2) or with KRP+DA (I_2 plus DA). I_1PRL secretion was significantly higher in 33-day-old control and melatonin treated (MEL) rats as compared to I_2 periods. However, in 31-day-old rats I_1 secretion was higher than in the I_2 or I_{2+DA} periods, in MEL rats. *In vitro* GH secretion was significantly higher at I_1 than during I_2 periods in the control 31- and 33-day-old groups, but not in MEL rats. The only significant effect of DA was the elevation of GH in prepubertal MEL rats. *In vitro* FSH release was increased by melatonin in 31-and 33-day-old female rats. No differences in PRL, GH and FSH secretion were found in adult rats. In conclusion, the results show that melatonin effects upon *in vitro* pituitary gland activity are reproductive-stage-dependent modifying the secretory capacity of the lactotrop, gonadotrop and somatotrop during prepubertal and pubertal ages but not in adult rats studied at a quiescent phase of the sexual cycle.

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INTRODUCTION

The pineal gland through its main hormone melatonin is involved in the modulation of reproductive activity in seasonal breeding mammals (Bittman et al. 1985, Hastings 1989, Misztal et al. 1996). By contrast, the influence of melatonin in reproduction of nonseasonal breeders is unclear (Arendt 1998). A major question that remains unresolved is the level of the neuroendocrine--reproductive axis at which melatonin has its effects. While the hypothalamus was considered initially to be the most likely site of this action, the discovery of a prominent population of melatonin receptors in the pars tuberalis of the anterior pituitary of the rat focused attention on this structure (Vanecek 1988, Willians 1989, Weaver and Reppert 1990). Several studies in the literature reflect the effect of melatonin on *in-vitro* secretion of pituitary hormones. Thus previous workers (Jetton et al. 1994) found that exposure of pituitary tissue from Golden hamster (Mesocricetus auratus) to melatonin using perfusion techniques, decreased the basal secretion of luteinizing hormone (LH) but did not affect the stimulated release of LH or follicle stimulating hormone (FSH) by gonadotropin releasing hormone (GnRH), regardless of the daily period of pituitary dissection. Other authors (Rozell and Mead 1993) showed that melatonin had no effect on pituitary response to GnRH and failed to inhibit prolactin secretion during a 48h culture period from pituitary of the seasonal breeding Spotted skunk (Spilogale pygmaea). However melatonin significantly reduced the inhibitory effects of dopamine (DA) on prolactin secretion.

Prolactin, FSH, and growth hormone (GH) are pituitary hormones implicated in reproductive function. FSH stimulates follicular development and estradiol synthesis (Leung and Amstrong 1980). GH administration to female hypophysectomized rats increased the esteroidogenic response to FSH *in vitro*, whereas reduced serum GH levels in intact rats produced a diminished ovary response to gonadotropins (Davoren and Hsueh 1986). In addition, delayed puberty in humans associated with a GH deficit, was reversed after GH therapy (Tresguerres 1995).

In the present work, we examined the possible direct effect of melatonin treatment on *in-vitro* pituitary gland hormones (FSH, GH, prolactin) secretion. This study was performed at three different reproductive phases, prepubertal, pubertal and adulthood in order to investigate possible age dependent changes. Having in mind that hypothalamic dopaminergic pathways have been considered as one pausible mechanism by which melatonin can act on the neuroendocrine-reproductive system (Tortonese and Lincoln 1995, Zisapel et al. 1995), the influence of DA on *in vitro* pituitary hormone (FSH, GH and prolactin) release was also studied.

METHODS

Experimental animals

Female Wistar rats from our colony were used. The animals were housed in a 12 h light/dark cycle (light on at 08.00 h) and at an ambient temperature of approximately 23°C, with standard rat chow and water ad libitum. According to Ojeda et al's classification (1980) concerning postnatal sexual maturation, the infantile period extends from 8 to 21 days, the juvenile or prepubertal from 21 to 32 and the pubertal from day 32 to the day of vaginal opening. Studies of female rats were started at either infantile, 20-day-old (n = 48), or adult, 60-day-old (n = 28). Female rats were submitted to melatonin or placebo treatment until different ages studied.

Melatonin administration

Melatonin was dissolved in absolute ethanol and diluted in 0.9% NaCl to a dose of 150 μg/100g BW. Melatonin was given s.c. 1,5 h before the end of the light phase, taking into account previous findings (Tamarkin et al. 1976). Both the melatonin (Sigma M-2550, Chemical Co, St. Louis, Mo) and vehicle solutions were made up fresh each day. Melatonin treatment started on day 20 and extended through the prepubertal period to day 31 (n = 12) or 33 (n = 10). In adults, melatonin treatment started at 60 days of age (n = 15) and was administrated for one month and then until first diestrus was observed. Control prepubertal (n = 12), pubertal (n = 14) and adult (n = 13) rats received only vehicle solution. Only rats not showing vaginal opening at 31 and 33 days of age were used in this study.

Pituitary incubations

The day after the end of the treatment period, animals were decapitated at 10 am. The in situ anterior pituitaries were removed, immediately dissected and divided in two halves to be used to analyze the gland functional ac-

tivity at two incubation periods: Basal rate release (I_1), the first hour of incubation, and the second incubation period (I_2), the second hour of incubation. The effect of DA, (Sigma H-8502 hydroxytyramine), (10^{-7} M), on the release of prolactin, GH and FSH was investigated. Hemipituitaries were placed in incubation tubes containing 1 ml of Krebs-Ringer phosphate (KRP) medium. They were incubated in a Haake SWD 20 bath at 37ºC, shaking at 60 cycles/ min, and were gassed with 95% O_2 : 5% CO_2. After 30 min, the medium was removed and discarded, and 1 ml of fresh medium was added. An hour later the medium (I_1) was removed and saved for measurements of the basal rate of hormone secretion during the 30-90 minute long first incubation period. It was then replaced with another 1 ml of KRP medium (I_2) or 1 ml of KRP plus DA (I_{2+DA}). One hour later, during the subsequent 90-150 minutes of incubation, the assay was ended. Both media I_2 and I_{2+DA} (second incubation periods) were removed and kept frozen for later measurement of hormones.

Hormone determinations

Hormones were measured by RIA employing second antibody facilitated separation with reagents kindly donated by the National Institute of Health (NIADD, Bethesda, MD). The assays were carried out as previously described (Esquifino et al. 1989a, Moreno et al. 1995). Values of prolactin were expressed as ng/hemipituitary/h of rat prolactin RP-3. The final dilution of anti-rat prolactin S-9 was 1:430.000. Values of FSH were expressed as ng/hemipituitary/h of rat FSH-RP-3. The final dilution of anti-rat FSH was 1:125.000. Values of GH were expressed as ng/hemipituitary/h of rat GH RP-3. The final dilution of anti-rat GH was 1:1.500.000. All samples were measured in the same assay in order to avoid interassay variation.

Statistical analysis

Statistical analysis was performed using the Sigma Statistics Program (Copyright Horus Hardware 1986). Results were expressed as mean ± SEM (Standard Error of the mean). Comparisons between groups at each incubation period studied, as well as among incubation times in the same group, were made by one-way analysis of variance (ANOVA). Individual comparisons were then made by Neuman-Keuls multirange test.

RESULTS

In vitro pituitary prolactin secretion

Basal prolactin release (I_1) increased from 31- to 33-day-old in to adult rats in both control and melatonin treated rats (Fig. 1). In 31-day-old melatonin treated rats,

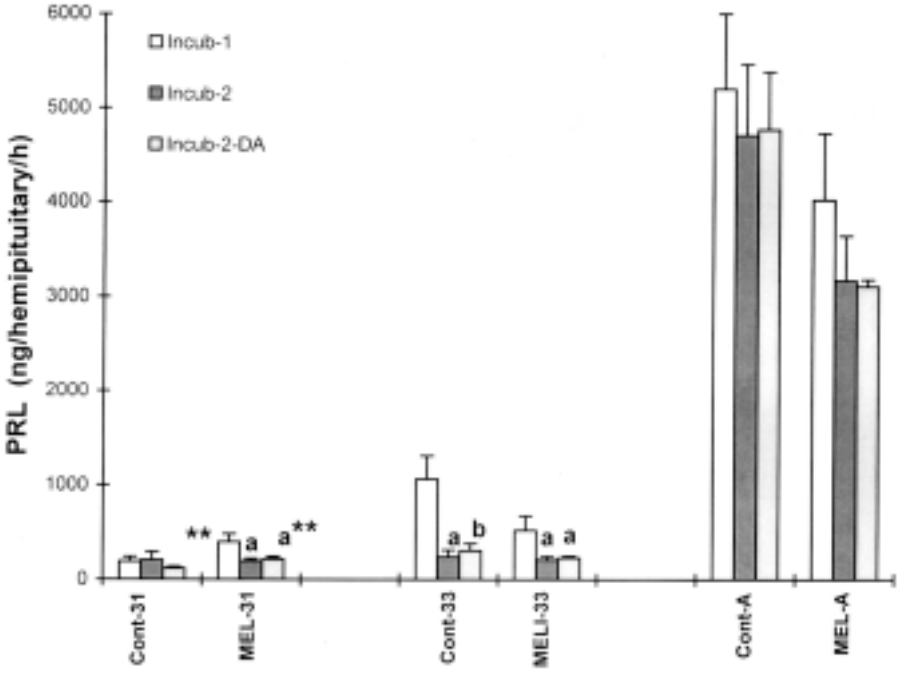

Fig 1. *In vitro* PRL secretion from the hemipituitaries of saline (Control) and melatonin treated (MEL) prepubertal (31 days), pubertal (33 days) and adult female rats. The concentration of PRL was determined in the medium collected from 30-90 min of incubation (Incub-1), and 90-150 min of incubation performed in the absence (Incub-2) or presence (Incub-2-DA) of 10^{-7}M dopamine. Results are expressed as mean ± SEM values of 8 to 15 independent incubations. The differences between the control and corresponding melatonin treated groups are indicated by asterisks: *, $P<0.01$; **, $P<0.05$; the differences from basal secretion within the group are indicated by letters: a, $P<0.01$; b, $P<0.05$.

basal secretion rate without (I_2) and with DA addition (I_{2+DA}) showed significantly increased ($P<0.05$) *in vitro* prolactin release as compared to control rats. However, in 33-day-old female rats, basal prolactin release was higher in control than in melatonin treated rats, although the differences were not significant. Prolactin release at both I_2 and I_{2+DA} incubation periods in 31-day-old control rats and 33-day-old control and melatonin treated rats, was significantly reduced ($P<0.01$, $P<0.05$) as compared to the basal incubation period (I_1). No significant differences were observed in adult rats at the diestrus phase between groups or incubation periods.

In vitro pituitary GH secretion

The results obtained for GH secretion rate (Fig. 2) were similar to those obtained for prolactin since basal GH release (I_1) increased from 31 to 33 days of age and in adult rats. In 31- and 33-day-old control rats, GH release was significantly reduced ($p<0.01$; $p<0.05$) at both I_2 and I_{2+DA} incubation periods as compared to the basal (I_1) secretion rate. For 31-day-old melatonin treated rats, GH release after DA addition (I_{2+DA}) was significantly higher ($P<0.05$) when compared to control rats. At 33 days of age melatonin treated rats' basal GH release was significantly lower ($P<0.05$) than that at the control group. No differences between groups or among incubation periods were observed in adult rats.

In vitro pituitary FSH secretion

In 31-day-old melatonin treated rats FSH release at both basal (I_1) and second incubation periods (I_2 and I_{2+DA}) (Fig. 3) showed significantly increased values ($P<0.01$) in comparison to control rats. In 33-day-old melatonin treated rats significantly higher *in vitro* FSH release was only observed at the basal rate (I_1) as compared to the control group. Lack of an effect of melatonin on *in vitro* FSH secretion in adult aged rats at the diestrus phase of the sexual cycle was observed. Contrary to results obtained for prolactin and GH release in prepubertal female rats, *in vitro* FSH release was not modified by the addition of DA (10^{-7} M) to the incubation medium (I_{2+DA}), when compared to basal release (I_1).

DISCUSSION

The results of this investigation clearly establish that pituitary prolactin release increased from prepubertal to adult age in both groups studied. Increased *in vitro* prolactin secretion with age is in agreement with previous data reported in plasma of female rats (Advis et al. 1981, Esquifino et al. 1989a, Moreno et al. 1995). *In vitro* prolactin secretion was more marked in 31-day-old melatonin treated rats than in controls. This difference disappeared in 33-day-old rats. This suggests that during the prepubertal period *in vivo* melatonin administration

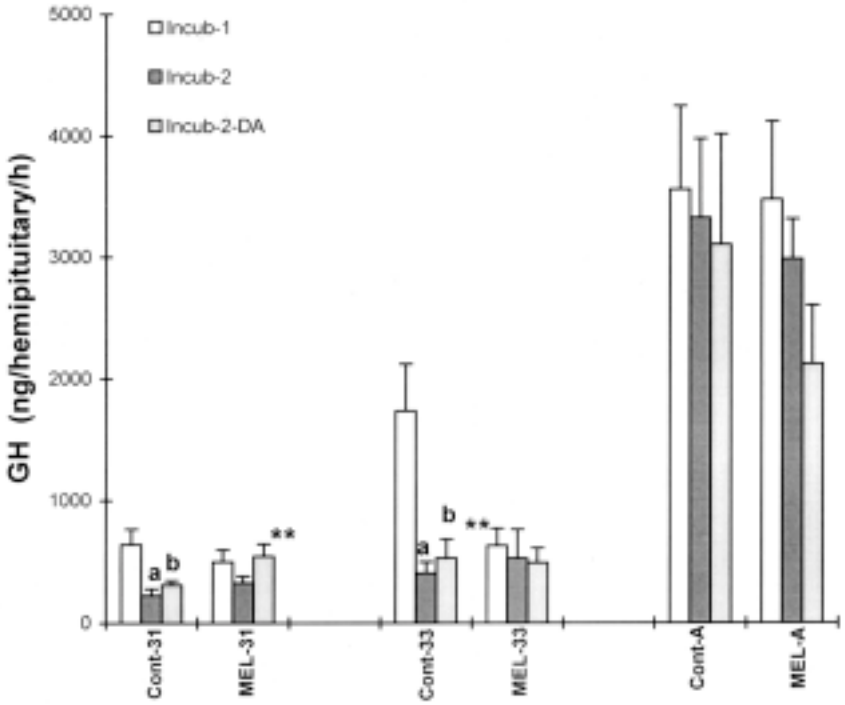

Fig 2. *In vitro* GH secretion from the hemipituitaries of saline (Control) and melatonin treated (MEL) prepubertal (31 days), pubertal (33 days) and adult female rats. The concentration of GH was determined in the medium collected from 30-90 min of incubation (Incub-1), and 90-150 min of incubation performed in the absence (Incub-2) or presence (Incub-2-DA) of 10^{-7}M dopamine. Results are expressed as mean ± SEM values of 8 to 15 independent incubations. The differences between the control and corresponding melatonin treated groups are indicated by asterisks: *, $P<0.01$; **, $P<0.05$; the differences from basal secretion within the group are indicated by letters: a, $P<0.01$; b, $P<0.05$.

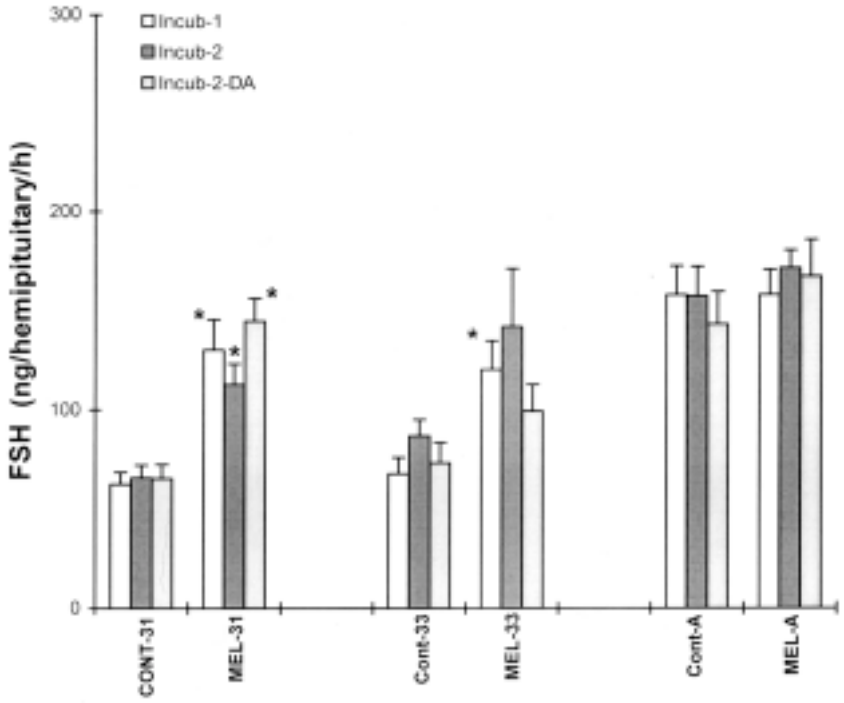

Fig 3. *In vitro* FSH secretion from the hemipituitaries of saline (Control) and melatonin treated (MEL) prepubertal (31 days), pubertal (33 days) and adult female rats. The concentration of FSH was determined in the medium collected from 30-90 min of incubation (Incub-1), and 90-150 min of incubation performed in the absence (Incub-2), or presence (Incub-2-DA), of 10^{-7}M dopamine. Results are expressed as mean ± SEM values of 7 to 14 independent incubations. The differences between the control and corresponding melatonin treated groups are indicated by asterisks: *, $P<0.01$; **, $P<0.05$; the differences from basal secretion within the group are indicated by letters: a, $P<0.01$; b, $P<0.05$.

slightly modifies the *in vitro* secretory capacity of the lactotroph. This is in agreement with the well known effect of this hormone on lactotroph activity (Waldhauser et al. 1987, Esquifino et al. 1989b). In pubertal 33-day-old female rats, as a consequence of higher basal secretion rates, prolactin secretion seem to decrease at second incubation periods, but not in 31-day-old control rats when basal prolactin secretion rate was lower. As this effect was not observed in adult rats, the results obtained during pubertal age suggest that when all the mechanism required for prolactin secretion are maturating, the stimulatory mechanism of prolactin is more active than the inhibitory, since prolactin secretion decreased a similar amount at the second incubation time independently of whether DA was added or not to the incubation media. The higher prolactin secretion observed in adult rats at all incubation periods are indicative of a pituitary gland in a consolidated hormonal stage. Lack of an effect of melatonin on these *in vitro* pituitary gland secretion rates are in agreement with results obtained from *in vivo* studies performed in adult female rats (Villanúa et al. 1989).

The results obtained for the *in vitro* GH secretion rates were similar to those observed for prolactin, since GH secretion rates increased from prepubertal to adult rats. This increase was blunted when melatonin treatment was extended until the prepubertal phase at 33 days of age. In this way, in blind or normal rats kept in constant darkness, in which melatonin secretion is increased, reduced pituitary GH content was found (Smythe and Lazarus 1973). However, this antisomatotropic activity of melatonin is controversial, and a facilitatory role of melatonin on basal GH release has been suggested at the hypothalamic level (Valcavi et al. 1993). The addition of DA to the incubation media did not result in reduced GH release from *in vitro* pituitary as was previously found (Hirasawa and Kamada 1992, Tuomisto and Mannisto, 1985, Moreno et al. 1995), but our results show that pituitaries from prepubertal rats primed with melatonin release far more GH than those from controls when DA were added to the respective media. This indicates a synergistic action of melatonin and DA only at this stage of sexual maturation. The reduced amounts of basal GH secretion observed in pituitaries from pubertal melatonin treated rats and the lack of an inhibitory effect at both the I_2 and I_{2+DA} incubation periods, contrary to what was observed in the control group, suggest a direct effect of melatonin on the somatotrophs. However melatonin exerted differential effects on both *in vitro* GH and prolactin secretory mechanisms depending on the sexual maturation of the female rats. No differences were observed in GH *in vitro* secretion from adult pituitaries of control and melatonin-treated groups. Similar effects depending of the age of animals were previously observed when the effect of cyclosporine treatment on *in vitro* prolactin and GH release was investigated (Moreno et al. 1995).

The results concerning FSH indicated that *in vivo* melatonin treatment modified basal *in vitro* FSH release in prepubertal and pubertal female rats. The observed influence of melatonin on *in vitro* pituitary FSH release disappeared in adult rats, at the diestrus phase, coinciding with previous results (Esquifino et al. 1989). This suggests that melatonin exerts modulatory effects at the pituitary level mainly in immature rats as was previously shown (Martin and Sttaler 1982). Opposite to what was observed for *in vitro* GH or prolactin release in prepubertal and pubertal female rats, pituitary *in vitro* FSH release was not decreased at the second incubation period independently of DA addition to the incubation media. It was previously found in rat studies that DA alone was able to suppress *in vitro* basal hemipituitary LH and FSH release when relatively high concentrations (30, 60 and 90 μmol/L) of DA were used (Karanth et al. 1992). The lack of a DA effect on *in vitro* FSH release may also be interpreted from the point of view that gonadotrop cells separated from normal hypothalamic control became unresponsive to external stimuli as was found for *in vitro* pituitary-hypothalamic interactions and the importance of their intact neural connections (Dutt et al. 1986).

An age dependency of melatonin treatment on *in vitro* pituitary gland hormone secretory capacity is deduced. During prepubertal and pubertal age secretions were influenced by melatonin, but no influence was observed in adult rats, at the diestrus or quiescent phase of the sexual cycle.

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