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Effect of Continuous Light and Melatonin on the Sexual Maturation of the Female Rat

Melatonin (MEL) treatment during the prepubertal period has been pointed out to produce a delay of the spontaneous vaginal opening time and of the initiation of the first estrous cycle (7). However a slight effect or lack of effect of MEL on sexual maturation of the female rat has been reported (1). MEL peak is involved in sexual maturation; when pinealectomized hamsters were given twice daily injections of MEL, the gonads were reduced (4). The present investigation aims at elucidating the effect of MEL alone on sexual maturation of female rat, when the source of pineal MEL was disrupted by light (3).

Female Wistar rats, maintained under 12:12 L/D at 23 ± 0 °C during pregnancy were used. Pups were selected from birth to form the following groups: A) Control. Pups maintained under 12:12 L/D, (lights on at 08.00 h) until the end of the experiment at vaginal opening day. B) Continuous light (L-L) from birth until the vaginal opening day. C) Pups submitted to the same experimental conditions as group B and MEL treated (125 µg/100 g B.W.) once a day, by s.c. injection at noon. MEL treatment started on day 21 until the vaginal opening day (5), in all groups. D) Pups maintained under the same conditions as group B, with two s.c. MEL injections (125 µg/100 g) with an interval of 6 h, the first injection at noon. E). Pups maintained under the same regime as group A, and receiving one s.c. MEL injection (250 µg/100 g) per day, at the end of the light phase.

Estrous phase was observed by vaginal smear on the day of vaginal opening. Rats were sacrificed by rapid decapitation between 16.00-17.00 h. Trunk blood was collected and plasma was stored at –20 °C for subsequent hormonal determination of LH by specific RIA (8). Body weight, pineal gland and ovary weights were recorded. Significant differences among the experimental groups were determined by two-way variance analysis (ANOVA).

Body and pineal gland weights were similar among the groups studied. Delayed vaginal opening was observed in CON + MEL treated rats, as compared to the other groups (table I). Advance vaginal opening time was observed in L-L + 2MEL as compared to the L-L group. The groups of rats showing earlier sexual maturation L-L + MEL and L-L + 2MEL

Table I. Vaginal opening (V.O.) day and ovarian weight of female rats.
Groups: A) Control; B) L-L, rats under constant light from birth; C) L-L+MEL, rats under constant light plus melatonin (125 µg/100 g B.W.); D) L-L+2MEL, rats under constant light plus 2 MEL melatonin (125 µg/100 g B.W.); E) CON+MEL, control rats plus melatonin (250 µg/100 g B.W.).

Groups	V.O. (days)	Ovarian weight (mg)	Ovarian weight (mg/100 g B.W.)
A) Control	37.57 ± 0.67 (21)[a]	53.72 ± 2.91 (21)	48.07 ± 2.10 (21)
B) L-L	38.19 ± 0.61 (26)[bc]	48.06 ± 2.59 (24)	43.46 ± 1.98 (25)[d]
C) L-L + MEL	35.42 ± 0.61 (14)[b]	53.30 ± 5.03 (14)	50.78 ± 3.98 (14)
D) L-L + 2MEL	35.30 ± 0.66 (13)[b]	61.13 ± 3.51 (12)	54.72 ± 2.71 (12)
E) CON + MEL	41.11 ± 0.72 (20)	53.30 ± 3.03 (20)	43.17 ± 2.48 (20)[d]

Values are mean ± SEM. In parentheses number of cases. [a] $p < 0.05$; [b] $p < 0.01$vs E; [c] $p < 0.01$ vs D; [d] $p < 0.05$ vs D.

showed higher relative ovarian weight associated with the highest LH values and higher percentage of proestrous-estrous smears. Plasma LH levels, were significantly higher in the L-L + MEL group (1.01 ± 0.18 pg/ml, n = 12) having significant differences ($P < 0.05$) as compared to the L-L group (0.59 ± 0.07 pg/ml, n = 12). Higher LH levels, and earlier vaginal opening time are associated with a higher incidence of proestrous-estrous smears at the vaginal opening day in the L-L + MEL (61.54 %) or L-L + 2MEL (78.95 %) groups. With significant differences ($P < 0.005$) in the smears between the L-L + 2MEL and the L-L group (36 %). Smears from vaginal epithelium indicate its response to estrogenic hormones (6). The increased percentage of proestrous-estrous smears observed in the L-L + 2MEL group could be interpreted as an index of agonist function of MEL on steroid hormones. Similar effect on estrogen levels has been observed using a small dose (5 µg/day) of MEL administered since day 23 of life, without significant effects on vaginal opening time, but with significantly increased uterine weight at 36-37 and 40 days (2). This suggests that estrogen levels were increased by MEL treatment at this time. When MEL (100 µg/rat) was injected, 3 h before darkness, on day 5 of life an increase in the number of estrous smears was also observed (8). Ovarian relative weight was the smallest in the group with delayed sexual maturation (CON + MEL).

Similarly MEL (100 µg) injected from day 15 of life, to rats under 14:10 L/D or 12:12 L/D, delayed the timing of vaginal opening accompanied by significantly decreased ovarian weight while the earlier age of vaginal opening was associated with increased ovarian weight (1).

These results confirm a role for MEL in the sexual maturation of the female rat, indicating a different sensitivity to MEL under constant light as compared to under diurnal photoperiod, as it is deduced from its inhibitory action and from the advance of the onset of sexual maturation under constant light.

Key words: Melatonin, Continuous light, LH, Vaginal opening.

Palabras clave: Melatonina, Luz continua, LH, Apertura vaginal.

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