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EFFECTS OF PHOTOPERIODS ON LH AND PROLACTIN (PRL) PLASMA LEVELS IN THE MALE RAT

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SUMMARY

The influence of different schedules of constant light (L/L) on plasma LH and prolactin (PRL) levels was studied. Control 102 days old male rats subjected to a light–darkness schedule (12 h light) and experimental rats submitted to 25, 45, 65 or 85 days of L/L were used. Compared with its controls, the groups submitted to L/L showed: higher LH (with 25 days L/L) higher PRL (with 25 and 65 days L/L) and lower LH and higher PRL levels (with 85 days L/L). To determine whether the age of the animal is related to the modifications induced by L/L, 65 days old rats submitted to a light–darkness schedule as well as L/L from birth were used. Results showed an increase in plasma PRL and also in plasma LH. These two groups showed a similar response to castration. The data indicate that to evaluate the effects of L/L it is necessary to take into account the duration of the L/L period and the age of the rats at which this period is started.

INTRODUCTION

The light–darkness period plays an essential role in physiology of the hypothalamus-pituitary-gonadal axis. Constant light induces precocious puberty in the female rat [1] with persistent vaginal cornification and absence of spontaneous ovulation [2, 3]. These animals also show reflex ovulation [4] and modifications in pituitary LH content [5]. The time of constant light necessary to produce these modifications has been shown to be age dependent [6].

Gonadal function of the male is also affected by a light–darkness schedule: In some species, an increase in the light period is associated with high prolactin (PRL) levels [7]. In the male rat constant light induces a decrease of plasma PRL levels with a modification of its circadian rhythm [8–10].

In the female rat, alterations induced by constant light are correlated with the time of constant illumination [3]. The aim of the present paper is to study the chronological modifications in LH and PRL plasma levels of male rats under constant light.

MATERIAL AND METHODS

In the first experiment, male Wistar rats under different light conditions were used. They were divided into five groups, submitted to:

Group 1. A 12 h light–darkness schedule (lights on 7.00 h, lights off 19.00 h)
Group 2. 25 days under constant lighting conditions (from day 77 to day 102 of age).
Group 3. 45 days under constant lighting conditions (between days 57 and 102 of age).
Group 4. 65 days constant light (from day 37 day 102 of age).
Group 5. 85 days under constant light (between days 17 and 102 of age).

On day 102 a single blood sample was taken from every animal at 10.00 a.m.

In the second experiment, two groups of male rats were used:

Group 6. Under a 12 h light–12 h darkness schedule from birth.
Group 7. Under constant light from birth.

On day 65, a blood sample was taken at 10.00 a.m. Three different subgroups were formed in order to obtain blood samples at different times after castration (days 1, 5, 9, 13 and 18; 2, 6, 10, 14 and 19; 3, 7, 11, 15 and 20) to analyse the participation of testicular secretion in the modifications induced by constant light.

Blood samples were drawn from the jugular vein under light ether anaesthesia. Blood was taken into heparinized tubes, centrifugated at 3.000 rev./min for 20 min at 4°C and plasma was kept frozen until use.

Plasma LH and PRL were measured in duplicate by a double antibody R.I.A., using kits supplied by the NIAMD. Highly pure preparations of LH and PRL (NIAMD-Rat-LH-I-4 and NIAMD-Rat-PRL-I-4 respectively) were labelled with ^{125}I utilizing the chloramine T method (11). The antibody for LH determinations was used at a 1/15.000 dilution and for PRL at a 1/2.500 dilution.

LH and PRL values are expressed in ng/ml of both reference preparations: NIAMD-Rat-LH-RP1 and

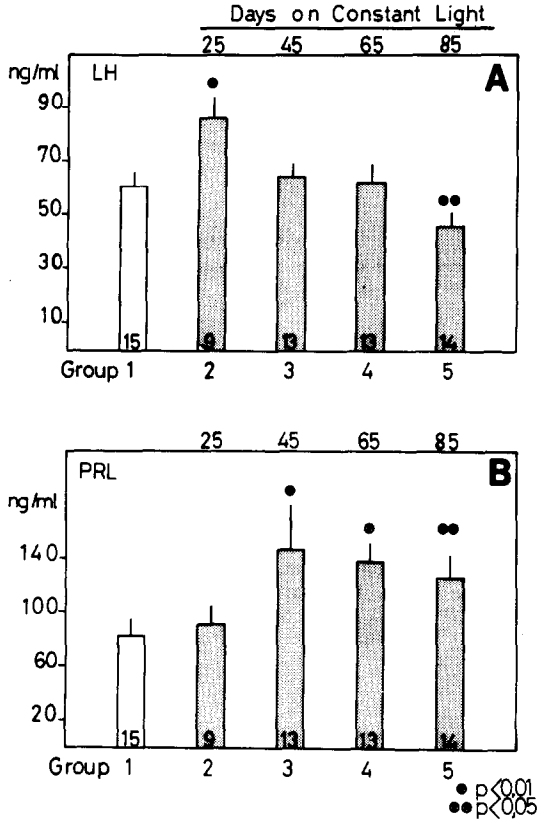

Fig. 1. Plasma LH values (Panel A) and PRL levels (Panel B) ($\bar{x} \pm$ SEM) in males submitted to light–darkness (group 1) and different periods of constant light (groups 2–5). Within bars number of cases.

NIAMD-Rat-PRL-RP1 respectively, Intraassay variations were 7% (for LH) and 9% (for PRL). Interassay variations were 10% (for LH) and 13% (for PRL).

Statistical analysis of results was performed by the Student's *t*, variance and Pairwise tests [12].

RESULTS

Effect of different constant lighting periods on plasma LH and PRL

Figure 1A shows the plasma levels of LH in groups 1 to 5. Animals of group 2 (submitted to 25 days of constant light) have higher values than those of group 1 ($P < 0.01$) whereas group 5 (85 days of constant light) has lower LH levels ($P < 0.05$). No differences can be seen between groups 1, 3 and 4.

In figure 1B PRL levels of the five groups are shown. The values found in group 2 are similar to those found in group 1. Groups 3, 4 and 5 have higher levels of PRL.

Figure 2 shows the LH and PRL plasma values in groups 6 and 7. The animals of group 7 (under constant light) have higher levels of both hormones ($P < 0.01$) compared to those of group 6 (under a 12 h light–darkness schedule).

Evolution of plasma levels of LH and PRL after castration

LH levels rise from the first day after castration in both groups with similar kinetic patterns although on days 11, 15, 19 and 20 after castration, higher values are observed in the animals under constant light (Fig. 3A). PRL levels (Fig. 3B) drop in both groups after castration (group 6: $F = 5.97$; $P < 0.01$ and group 7: $F = 11.5$; $P < 0.01$).

DISCUSSION

From these data it is easy to observe that constant light modifies LH and PRL secretion in the male rat. These modifications are dependent upon the length of the constant light period and also partially upon the age of the animals in which the constant illumination periods were started.

Plasma levels of LH are high in short periods (25 days) and low in long periods of constant illumination (85 days). Sixty-five days of constant light from the time of birth induce a rise in plasma LH whereas no modifications can be observed in older animals exposed for the same length of time to constant light, but started on day 37 of age (see groups 4 and 6). Modifications of the synthesis and/or secretion of LH in the female under constant light have been described with a decrease in pituitary content of this gonadotrophin [3, 5].

Plasma levels of PRL are increased in the female rat rendered anovulatory under constant light [13, 14]. In the male, periods of 7–21 days of constant light decrease PRL plasma levels, increasing its pituitary content [8, 9] and reversing the nicthameral rhythm [10]. In our data, prolonged periods of constant light (45–85 days) induce, on the contrary, an increase in plasma levels of PRL. No clear explanation for this difference is found: Melatonin or sero-

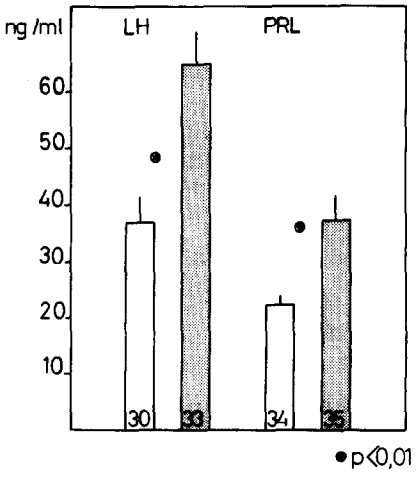

Fig. 2. Plasma LH and PRL values ($\bar{x} \pm$ SEM) in males subjected to light–darkness (Open bars; group 6) and to constant light (Filled bars; group 7).

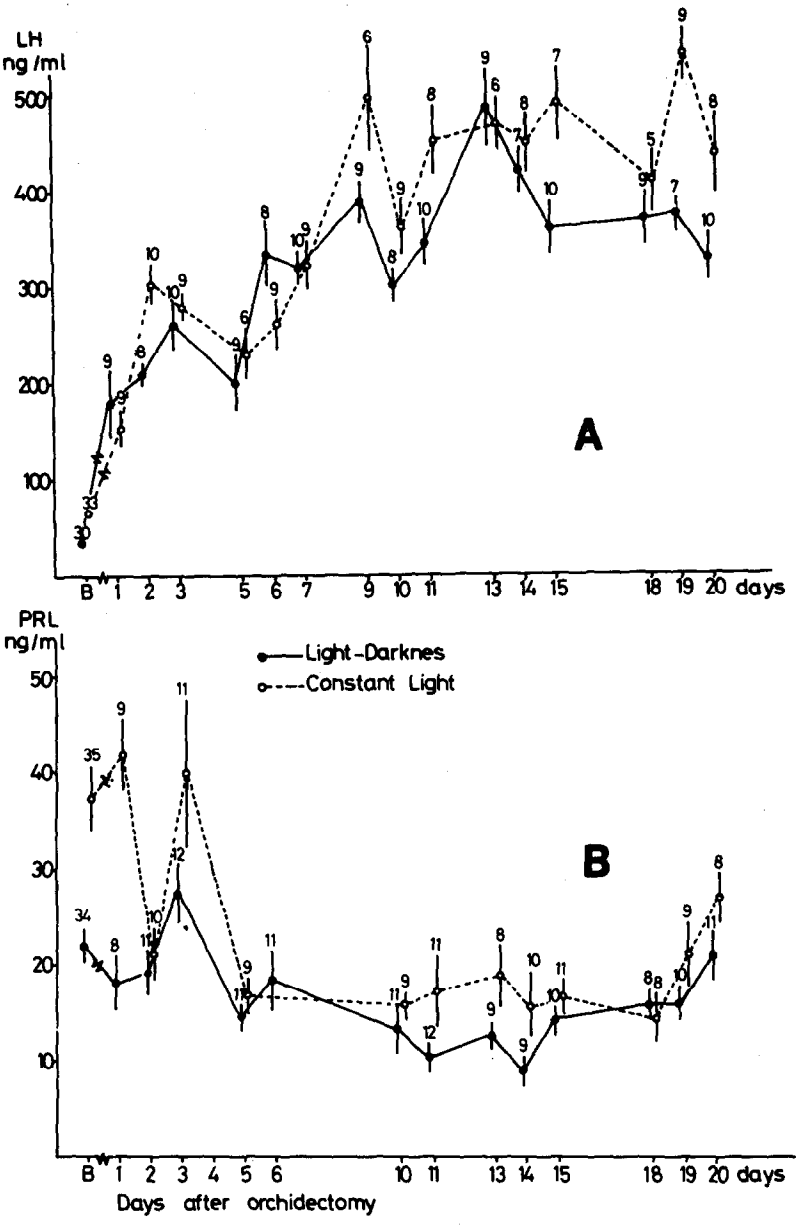

Fig. 3. Plasma LH values (Panel A) and PRL values (Panel B) ($\bar{x} \pm$ SEM) on different days after castration, in males submitted to light–darkness and to constant light from birth (groups 6 and 7). Number of cases is shown near every data.

tonin increase PRL secretion in male rats [15] and during constant light melatonin synthesis by the pineal gland is inhibited [16]. This can explain the PRL decrease found by other authors [8] and its reversibility after pinealectomy [9]. On the other hand, catecholamines block PRL secretion, and drugs interfering with synthesis enhance plasma levels of this hormone [17, 18]. Constant light seems to increase hypothalamic levels of serotonin and decrease those of catecholamines [13]. Several mechanisms seem to be involved in PRL secretion changes during constant light. Knowledge of the complex regulatory mechanism may provide an explanation for the high PRL levels found in prolonged periods of constant light.

Considering the simultaneous evolution of LH and PRL related to lighting period, compared with its controls (group 1), several patterns are found: high LH levels with normal PRL levels (group 2), high PRL levels with no modification of LH levels (groups 3 and 4) and both higher LH and PRL levels (group 7). These differences suggest the convenience of making chronological studies for a better understanding of the modifications as has been postulated for the females [3]. Many authors [19–21] indicate the possibility of an inhibitory effect of PRL on LH levels, as

shown in group 5, but there is no explanation for the simultaneous elevation of LH and PRL found in group 6. The high values of PRL found in this group contrast with the non modified values appearing in animals of 102 days of age submitted to the same constant light period. These results suggest a major role of animals age, as described formerly for female rats [6].

After castration, plasma LH increase and PRL decrease, as has been described elsewhere [22]. The evolution after castration seems to be independent of light, although for plasma LH a tendency to higher values under constant light is observed 2 weeks after orchidectomy.

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