Research Article

Effects of long-term exposure to light or darkness and return to normal light-dark cycle on serum melatonin levels in rats

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ABSTRACT

Increasing evidence suggests that the presence of constant light or darkness have diverse effects on circadian physiology. The aim of this study is to explore serum levels of melatonin upon return to a normal light-dark cycle (LDC) in rats exposed to constant light (LL) or darkness (DD). Results showed the different profiles of melatonin levels after exposure to LL or DD. Similarly, the restoration of the LDC (12L:12D) modified the endogenous melatonin levels. In the LL group, serum melatonin remained at levels similar to control values, and when normal LDC was restored, melatonin levels of the rats decreased but without significant difference compared to control. In the DD group, serum melatonin increased significantly (22%), and upon switching to normal LDC, serum melatonin level was significantly decreased compared to constant dark condition. Even 15 days later, a significant 76% drop in serum melatonin level was still observed in LDC condition. The results suggested that prolonged exposure to LL or DD, especially to DD, had profound effects on the serum melatonin. LL has little influence and this result can be explained by the potential compensation of extrapineal melatonin generated by other tissues and organs.

Key words: Melatonin, circadian rhythm, light exposure, constant darkness, rat

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1. INTRODUCTION

The physiological activities and patterns of animal behavior are highly regulated by circadian rhythms (1, 2). The fundamental adaptive function of an endogenously programmed rhythmicity is to provide an optimal and anticipatory temporal organized physiological processes and behavior to fit the environment (3). Synchronization of endogenous circadian rhythms to the 24-hour environmental alteration in organisms occurs through the daily adjustment of the internal clock by external time cues (zeitgebers). The most important and reliable cue for this synchronization is the daily light-dark cycle (LDC) (4). Light sensing by the retina plays a key role in animals and human physiology by relaying information about the time of the day and the seasons of the year. Increasing evidence suggests that the constant light (LL) or darkness (DD) exposure in vertebrates can disrupt many aspects of circadian rhythm and have diverse effects on physiology, neuroendocrine and behavioral regulation of them (5-

8). Thus, numerous studies have been conducted exposing laboratory animals to free-running conditions of LL or DD for a period of several days. For example, the circadian release of hypothalamic thyrotrophin-releasing hormone (TRH) of rats is disrupted after exposure to LL or DD (9). In addition, a decrease in thyroid activity and a notable decrease in gonadal activity were observed in male rats exposed to DD compared to animals exposed to a normal LDC (10). In female rats, the duration of gestation is shortened after exposure to DD compared to the exposure to normal LDC (11) and natural tendency of deliver during the daytime was completely abolished under LL conditions. Also in rats, long-term exposure to DD resulted in the development of a depression-like behavioral phenotype sensitive to desipramine (12). This depression-like behavioral phenotype was also observed in mice and leads to reduced hippocampal progenitor cell proliferation of these mice when they were exposed to the DD compared to the normal LDC (13).

In most mammals, circadian rhythms are synchronized by the LDC via entrainment of the suprachiasmatic nucleus (SCN), representing the master circadian pacemaker (14). Because the SCN is neurally connected to the pineal gland, the LDC also regulates the biosynthesis of melatonin (15, 16). This rhythm has been proposed as a link between photoperiod and observed effects on mammalian including human behavior and physiology (17-20).

Serum melatonin (ML) exhibits a circadian rhythm with high level at night and low level during the day, reaching peak level between 02:00 and 04:00 am. In rats, nocturnal restricted up-regulation of arylalkylamine N-acetyltransferase (AANAT) mRNA has a 150-fold increase in the pineal gland at night compared to the daytime (21). Because pineal ML production occurs during the dark phase and is acutely suppressed by light (22), and also, since ML is quickly cleared from the circulation following its release from the pineal gland, the time and duration of the ML peak reflect the environmental night period (16). Therefore, the long nights result in a longer duration of ML secretion (23).

There are also sources of extrapineal ML production, mainly in the Harder gland, retina, skin and intestine, among others (24, 25). The ML levels in these tissues seem to be higher than the pineal generated ML in serum (26).

Light exposure at night is the main factor involved in the suppression of pineal ML production in vertebrates, and the retino-hypothalamic tract is involved in this action, reducing pineal activity (27). Previous studies have confirmed that pineal ML synthesis and secretion decreased under LL (28-30) and it has the same effect as surgical removal of the pineal gland (31). Serum ML levels of pregnant rats exposed to LL were significantly lower than those from the control group (12L:12D) (32). On the other hand, exposure to DD contributes to a free-running rhythm in which serum ML levels are increased (30, 33).

In view of the effects of alterations related to the photoperiodic changes on ML production, the aim of this study is to explore serum levels of this hormone of rats exposed to LL or DD for a long period of time (15 days) and then returned to normal LDC. To the best of our knowledge, this study is the first to demonstrate such effects. Our hypothesis is that the circadian system involved in the ML production, affected by free-running light conditions, will differently respond according to constant light or darkness for a long time exposure.

2. MATERIALS AND METHODS

2.1. Animals.

Twenty-five male Sprague-Dawley rats, weighing between 230 ± 15 g, were acclimated for 2 weeks at 22º ± 2º C room temperature, and under normal LDC of 12L:12D (light on: 8:00 am) before the experiment.

2.2. Experimental protocol.

Rats were randomly divided into five experimental groups, with five animals per group, and were placed in cages provided with food and water *ad libitum*.

To evaluate the effects of lighting regimens on ML plasma levels, one group was considered as control (CG) and was exposed to LDC 12L:12D for 15 days; two groups were exposed to a free-running regimen of 24 hours of light (LL), and two groups were exposed to a free-running of 24 hours of darkness (DD). After said period, animals were returned to LDC for another 15 days (LL/LD or DD/LD groups).

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Morón (Acta Nº 1, 21/03/2016). Experimental animals were treated according to Canadian Council on Animal Care (https://www.ccac.ca/en/standards/guidelines/) as well as according to ARRIVE guidelines (https://arriveguidelines.org/).

2.3. Treatment of rats with different light exposure schedules.

The two LL groups were kept in an environment illuminated with two fluorescent tubes (Philips TL-D 36W 840 Super 80; light color: 4000K - cold white; luminous flux: 3350 Lm). Rats were kept and exposed to constant light (280 lux, TES model 1330 luxmeter) in their cages for 15 days. 15 days after treatment, 5 mL of blood samples were taken from the rats in one of the LL groups. Rats in the other LL group were exposed to another 15 days under normal photoperiod 12L:12D (LL/LD group). At the end of this period, 5 mL of blood samples were taken from these rats. The two DD groups were kept in constant darkness for 15 days. The cleaning of the cages, feeding, and handling of the animals were carried out using dim red lights (incandescent lamp, 25W) placed 3 m away from the animals. 15 days after treatment, 5 mL of blood samples were collected from the rats in one of the DD groups. Rats in the other DD group were exposed to another 15 days under normal photoperiod 12L:12D (DD/LD group). At the end of this period, 5 mL of blood samples were taken from these rats.

2.4. Preparation of blood samples.

Considering the previous information regarding the influence of the photoperiod on circadian rhythms we standardized blood sampling in all experimental groups. The blood was collected during the lighting phase of the LDC, at 4 pm (zeitgeber time = ZT8, were ZT0 is the time of lights on).

After each period necessary for the evaluated treatments to take effect, blood samples were taken from anesthetized animals in a CO_2 chamber. Five mL of blood was taken from their hearts, collected in a tube and stored at room temperature for 10-20 min to be clotted. After centrifugation at 3000 rpm for 10 min, the serum was collected and stored frozen at -20º C for analysis.

All the procedures described above were performed under dim artificial light.

2.5. Melatonin measure in serum.

Serum ML levels were measured using the ELISA assay (Melatonin ELISA Kit (ab213978), Abcam), according to the instructions provided by the manufacturer.

2.6. Statistical analysis.

One-way ANOVA test and *post-hoc* Multiple Comparisons versus Control Group (Dunnett's Method) were used for analysis (SigmaStat 3.5, Systat Software, Inc). All data were expressed as mean and standard deviation (S.D.); $p < 0.05$ was considered as the significant level.

3. RESULTS

Multiple comparisons were used to compare alterations of serum ML levels between groups. The results showed that rats exposed to the constant light for 15 days (LL group) had a slight increase in serum ML but without significant difference compared to the CG ($p > 0.05$). When the LL rats was switched to the to normal LDC (LL/LD group) the serum ML level was significantly reduced (26%) compared to the LL group but had no significant difference compared to the CG group (16 % less than CG) (Figure 1). In contrast, the serum level of ML in DD group was significantly increased (22%, $p < 0.05$) compared to the CG group. When the DD rats were returned to normal LDC for 15 days (DD/LD group), surprisingly the serum ML in DD/LD group was significantly lower than that in CG group (24% less) ($p < 0.05$) (Figure 1).

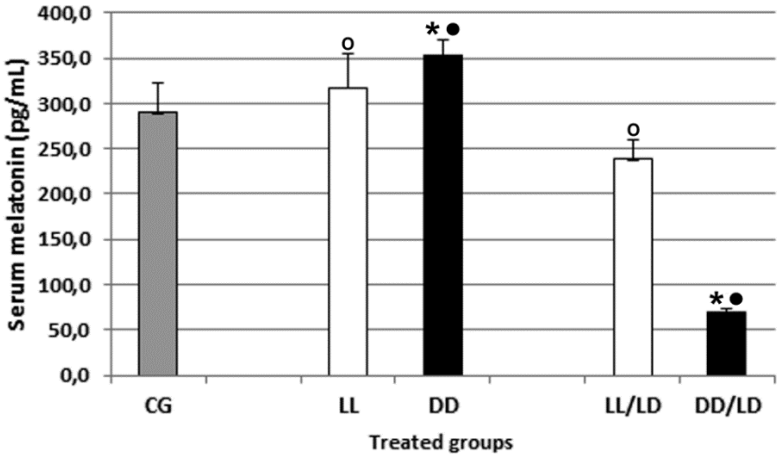

Fig. 1. Effects of different light exposures on the serum melatonin levels.

*Data were expressed as mean ± S.D (n = 5). CG, control group 12:12 h L:D; LL, constant light exposure (15 d); DD, constant dark exposure; LL/LD, constant light exposure returned to normal light-darkness cycle (15 d); DD/LD, constant dark exposure returned to normal light-darkness cycle (15 d). * DD or DD/LD vs CG $p < 0.05$; ᵒ LL vs LL/LD $p < 0.05$; • DD vs DD/LD $p < 0.05$.*

4. DISCUSSION

Our results further confirmed that serum ML level could be influenced by the photoperiodic manipulations in animals including rats. Serum ML mainly reflects the activity of pineal gland and it is well documented that light, particularly the blue light suppressed the pineal function to reduce serum ML level (34, 35). The different serum ML levels were reported in rats. For example, some studies showed serum ML level as high as 4000 pg/mL (36), but others reported to be 461 pg/mL (37) in mid-light detected using Abcam ELISA kits.

In our study, the serum ML level was 289 ± 32.7 pg/mL in the CG group. This value was consistent with reports from several groups. Therefore, such values and their variations according to the treatments in our study were consistent with those observed by Farhadi *et al*.

(33), Honma *et al*. (38) and Fukuhara *et al*. (39). Thus, using this value as the baseline level in rats exposed to the normal LDC (12L:12D) enable us to compare changes in serum ML levels by manipulations of photoperiod length.

The studies of rats exposed to prolonged LL obtained controversial results as to the serum ML levels at the zeitgeber time 8 (ZT8). After exposure to the constant light for 15 days, the serum ML level of rats was not decreased but was slightly increased ($p > 0.05$) compared to the CG group. The results was similar to those of Farhadi *et al*. (33), who reported that constant light exposure for 10 days did not change the serum ML levels in rats. In addition, these authors recorded an increase in the blood ML concentration with oral administration of ML in LL condition. In contrast, some studies had reported that animals under constant illumination for one or two weeks reduced their serum ML levels in chickens (40), rats (30, 31) and hamsters (41). The data obtained by Escribano *et al*. (30) showed a significant drop of ML levels in serum, tissue and pineal gland of rat under LL compared to the CG.

Since the serum ML level is dependent on the pineal function, therefore, the morphological alterations of rats pineal gland under constant light exposure were analyzed. For example, Mochizuki et al. (42) observed minimal effects of the LL exposure for 10 days on the histological structure of the pineal gland. In another study, 45 days of LL reduced the volume of cell organelles in the pinealocytes, while most of these changes returned to a normal condition after 90 days of LL (43). Logvinov *et al*. (44) demonstrated a decrease in the number of organelles of pinealocytes during the initial hours of LL. However, the pineal secretory activity increased after 24 hours later, while the cellular structure and general structure of the gland returned to normal until 30 days later.

In the current study, no significant changes of the serum ML level was observed when the animals were exposed to the LL for 15 days or they were returned to the normal LDC compared to the CG . The lack of response of serum ML on long term of LL exposure could be explained by the following reasons: 1) the term of LL exposure does not affect pineal function, and 2) the extrapineal ML generated by Harder gland, retina, skin, intestine and others compensated the serum level of ML (18, 33).

The consequences of exposure to long-term of constant darkness in behavior and neural systems have been reported for laboratory animals (12, 45). Rats under constant darkness for several weeks exhibited distinct behavioral and anatomical features matching to the characteristics of human depression (8). In humans, the circadian system is usually disturbed during the polar winter, largely due to insufficient bright light exposure (46). This perception was reinforced when seasonal affective disorder (SAD) was classified as an illness in 1984. This disorder, which is characterized by recurrent episodes of major depression with a distinct seasonal pattern, was attributed to long winter nights and could be successfully treated with extra bright light, which mimics a long summer day (47, 48).

Regarding the effects of constant dark exposure in rats, our study demonstrated a significant increase in serum ML levels (up to 22 %). Other researches in rats showed that a period of 10 days of constant dark exposure increased serum ML levels (30, 33, 49, 50). Generally, ML production by the pineal gland begins at the onset of the dark of the day (16) and longer the night, the more the ML production is (23). This was also confirmed in our study with the increased ML level in DD rats. However, once these DD exposed rats were returned to normal LDC (DD/LD group), ML levels were significantly decreased and was even lower than that in the baseline level of CG. The results suggest that after long term of DD exposure, the ML synthetic machineries of pineal gland and the circadian axis were more sensitive to the light exposure after returning to the normal LDC. This hypothesis should be tested in the future studies.

Overall, our results indicate that the prolonged period of dark exposure has more marked effects than that of prolonged light exposure.

Finally, it can be inferred from our results that manipulations of the photoperiod, if performed correctly, can modify the circadian system and modulate the endogenous production of ML in animals.

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AUTHORSHIP

COC contributed to the conception of this study and wrote the original draft of the article. JCP, MPH, and COC contributed with the experimental design and data acquisition and analysis. All authors contributed to data interpretation and critically revised the manuscript and approved the final version of the paper.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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