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Brief Communication

Effects of long-term light, darkness and oral administration of melatonin on serum levels of melatonin

Naser Farhadi [a], *Majid Gharghani* [a], *Zahra Farhadi* [b,*]

[a] *Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran*
[b] *Department of Biology, Shiraz University, Shiraz, Iran*

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ABSTRACT

Background: Continuous light or darkness has various effects on different systems. In the present research work, the effects of constant light and darkness exposure of male rats and oral administration of exogenous melatonin on the serum levels of melatonin have been studied.

Methods: Thirty adult male Wistar rats were divided into six groups of: (1) Control, (2) melatonin, (3) light, (4) light and melatonin, (5) darkness, and (6) darkness and melatonin. All groups were placed according to light conditions for 10 days. Melatonin was administered orally after a period of 10 days to Groups 2, 4, and 6 (10 mg/kg of body weight). Serum levels of melatonin were measured using ELISA.

Results: The results showed the significant difference on serum melatonin in darkness, no light, and control groups. Although serum levels of melatonin were different in melatonin groups, the difference is not significant.

Conclusions: We concluded that being exposed to continuous darkness leads to an increase in serum melatonin.

Pineal, a small gland located deep inside the brain, has been a topic of discussion for decades. Many physiologists have represented it as a member of the nonperformance organ or limited to the effects on sleep and sexual activity, or the place of the spirit. However, recently, researchers have considered its effects on various physiological procedures [1,2]. After the extraction of pineal melatonin by Lerner et al., new researchers have conducted studies in the field [3]. Melatonin, produced by the pinealocytes, enters the circulation as an endocrine hormone and binds to receptors on a variety of target tissues to exert their physiological responses [4]. Because pineal melatonin production occurs during the dark phase and is acutely suppressed by light, and also, since melatonin is quickly cleared from the circulation following the cessation of its production, the time, and duration of the melatonin peak reflect the environmental night period [5]. Plasma melatonin exhibits a circadian rhythm with high levels at night, and low levels during the day, attaining peak concentrations of plasma melatonin between 02:00 and 04:00 am. Longer nights result in the longer duration of melatonin

* *Corresponding author*. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Kohgiluyeh Va Boyer Ahmad, Yasuj, Iran. Tel.: +98 9178492605; fax: +98 7433235153.
E-mail address: naserfarhadi42@yahoo.com (Z. Farhadi).

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secretion [5]. Some studies have been carried out on various effects of long-term exposure to light and darkness. Exposure to continuous darkness shortened the duration of gestation [6]. The marked tendency to deliver during the daytime was not influenced by exposure to continuous darkness but was completely abolished by constant light [6]. A nyctohemeral variation of hypothalamic thyrotropin-releasing hormone (TRH) was observed in light–dark exposed animals, while this variation was abolished by exposure either to constant light or constant darkness. These data indicate that any study involving hypothalamic TRH determination should be consider the diurnal variation and the effect of environmental light exposure [7]. When compared with the rats exposed to normal lighting rhythm, continuous darkness exposure resulted in a decrease in the thyroid activity as well as a remarkable decrease in gonadal activity in male rats [8].However, what is the influence of continuous light, darkness and exogenous melatonin on serum levels of melatonin? Is it similar to circadian rhythm or not? In the current study, we are going to answer this question.

Methods

In this study, 30 adult males Wistar rats weighing between 250 and 300 g went under the tests. Rats were randomly divided into 6 groups of 5 and were placed in special cages. For compatibility with the new environment, rats were under normal condition of light and darkness for a week (12:12) and the temperature range of 22–24 °C with free access to water and food. They went under the tests considering an institutional protocol in accordance with ethical principles approved by the Ethical Committee at the Yasuj University of Medical Sciences.

Animals were categorized into six groups of: (1) Control, (2) melatonin, (3) light, (4) light and melatonin, (5) darkness, and (6) darkness and melatonin. All groups were placed according to light conditions for 10 days.

Melatonin

Melatonin was administered orally after a period of 10 days to Groups 2, 4, and 6 (10 mg/kg of body weight) [9]. Melatonin was prepared from Sigma–Aldrich (USA) in the form of 1 g powder dissolved in distilled water (2 mg/ml).

Preparation of blood samples

Rats were anesthetized and blood samples were taken from the heart, after 10 days in Groups 1, 3, and 5, and 1 h after the administration of melatonin in Groups 2, 4, and 6. All of samples were collected morning from 8 to 10 immediately after bring out from the experiment. Five milliliter blood was taken from the heart after anesthetization of the rats by ether, and spilled in the tube and stored at room temperature for 10–20 min to be clotted. Then, the centrifuge was performed (20 min, 2000–3000 rpm) and the obtained serum was poured in microtubes with lid and it was transferred into the 20 °C freezer so that all samples were collected and sent to lab for testing. Serum levels of melatonin were measured by using ELISA (Glory-Science USA).

ELISA method

ELISA was performed in 96-well plates and serum was incubated in a well. After a certain time, the serum was removed and weakly adherent antibodies were washed off with a series of buffer rinses. To detect the bound antibodies, a secondary antibody was added to each well.

Attached to the secondary antibody is an enzyme such as peroxidase or alkaline phosphatase. These enzymes can metabolize colorless substrates into colored products. After an incubation period, the secondary antibody solution was removed and loosely adherent ones were washed off as before. The final step was the addition of enzyme substrate and the production of the colored product in wells with secondary antibodies bound. When the enzyme reaction was complete, the entire plate was placed into a plate reader and the optical density was determined for each well. The level of the color produced was proportional to the level of the primary antibody bound to the proteins on the bottom of the wells.

Statistics

ANOVA and *post-hoc* Tukey's test were used for analyzing. All data were expressed as Mean and standard error. The median with minimum and maximum also presented and $p < 0.05$ was considered as the significant level.

Results

The results showed a significant difference in serum melatonin levels of the six subgroups [$p < 0.05$, Table 1]. One-way ANOVA test was used for comparing the subgroups of control (without melatonin) in three main groups (control, light, and darkness) to investigate the effect of light on serum melatonin. Results indicated significant differences between darkness, no light, and control groups [$p < 0.05$, Table 1, Fig. 1]. Thus, continuous darkness leads to increasing the melatonin serum levels. Furthermore, serum levels of melatonin were significantly different in light and dark melatonin groups compared to control group [$p < 0.05$, Table 1, Fig. 1].

Discussion

This study showed that continuous darkness (with and without oral administration of melatonin) caused an increase in serum melatonin levels. Results also demonstrated that long-term light with orally melatonin, not light alone, leads to an increase in serum levels of melatonin. One of the primary findings concerning the melatonin production in the pineal is that this process of production is initiated at the beginning of the evening and the peak occurs in the middle of the night [10]. However, what happens when the brightness or darkness lasts long? In a period of 45 days of continuous contact to light, the volume of nuclei, Golgi, and mitochondria decreased in the pineal gland cells. However, most of these changes returned to a normal condition after 90 days [11]. In another study on male rats, the effect of continuous light was