Neuroscience Letters 543 (2013) 78–83

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Negative impact of melatonin ingestion on the photopic electroretinogram of dogs

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HIGHLIGHTS

- Melatonin ingestion at high dose during the day decreases the photopic ERG a- and b-wave amplitude.
- The photopic flicker ERG amplitude is reduced following melatonin ingestion.
- In scotopic ERG, melatonin ingestion has no significant impact on a- and b-wave maximal amplitude.
- Melatonin ingestion at high dose during the day has no impact on a- and b-wave implicit time in photopic and scotopic conditions.

ARTICLE INFO

Article history:
Received 11 December 2012
Received in revised form 22 January 2013
Accepted 19 February 2013

Keywords:
Melatonin
Electroretinogram
Retina
Cones
Dog

ABSTRACT

Melatonin follows a circadian rhythm entrained by the light/dark cycle and plays a role in promoting light sensitivity at night. It has been suggested that melatonin and dopamine reciprocal inhibition may contribute to the switch between day and night vision. The purpose of this study was to investigate the impact of a high dose of melatonin administration on the photopic and scotopic electroretinogram (ERG) of dogs in the daytime, when it is not thought to be present. Photopic and scotopic ERG luminance response functions were obtained from 7 anaesthetized beagle dogs (3 males and 4 females), once without melatonin (control) and once after oral administration of melatonin (90 mg/dog). Vmax (maximal b-wave amplitude achieved) and log *K* (retinal sensitivity) were calculated from the derived luminance response function. Photopic flicker ERG was also recorded. In photopic condition, a-wave amplitude (control: −126.90 μV; with melatonin: −49.64 μV; $p < 0.001$) and Vmax (control: 252.50 μV; with melatonin: 115.40 μV; $p < 0.001$) were decreased. A significant reduction of the photopic flicker ERG amplitude was observed after melatonin ingestion. In scotopic condition, an overall difference was reported before and after melatonin ingestion for the a- and b-wave amplitude, but no change was significant for Vmax. Melatonin ingestion at a high dose during the day decreases the photopic amplitude of a- and b-wave, but has no impact on implicit time. This negative impact of melatonin on photopic system may serve to promote night vision.

1. Introduction

The light/dark cycle is known as the most important environment synchronizer of the circadian pacemaker in mammals. Melatonin is a highly conservated molecule present in all studied species [32] and it is considered as an internal *zeitgeiber* for the circadian system [21]. Its level is known to be high during the night and low during the day and its production can be suppressed by light exposure to the eyes [17]. This hormone is excreted for the most part by the pineal gland at night, but it is also produced in several structures of the eye such as the lens [33], the iris, the ciliary body [27], the lacrimal glands [22] and the photoreceptors of the retina [5]. Melatonin precursor, L-tryptophan, is also present in some foods [25] and it can be ingested, as in the present experiment. It is also commonly used as a chronic self-medication for sleep or circadian disorder treatment [15].

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http://dx.doi.org/10.1016/j.neulet.2013.02.070

Many studies raised the possibility of the existence of a biological clock in the eye. It has been demonstrated that melatonin and dopamine production as well as their reciprocal inhibition follow a circadian rhythm that appears to be entrained by the light/dark cycle and may contribute to the switch between day and night vision [26]. In fact, in the retina, dopamine production has been shown to be triggered by light and produced during the daytime [37] whereas melatonin secretion increases during the night darkness. Therefore, as dopamine and melatonin act as mutual antagonists [36], the production of retinal dopamine during the day seems to promote the cone system, as measured in the iguana [23], whereas the production of retinal melatonin during the night seems to favor the rod system, as measured in the chick [24].

The circadian changes in retinal function can be assessed using the electroretinogram (ERG) technique which allows direct assessment of cone and rod system by recording the light-evoked electric potential originating from the retina in response to standardized flash stimuli. It has been demonstrated that, in entrained eyes, the rod driven b-wave amplitude was lower in the morning [7,6]. A shorter b-wave implicit time was observed during the day, with no change in ERG amplitude in photopic condition [12,13]. A study also reported an increase of photopic b-wave amplitude at noon [19]. However, the origin of these changes is still unclear and it is suggested that dopamine and melatonin are implicated in these circadian changes [23,10,11,18,16,29].

A study by Gagné et al. [11] investigated the impact of oral melatonin on the human ERG cone response. It was demonstrated that the cone maximal response was decreased by about 8% and the b-wave implicit time lengthened following the ingestion of 15 mg of melatonin. This result was consistent with the suggestion that when melatonin is taken during the daytime, when it is thought not to be present, it acts as inhibitor of retinal dopamine which is a promoter of day vision through the cone system. Rod function was however not assessed and therefore it is not known if it would be affected by the presence of melatonin. The goal of this experiment was to investigate the impact of oral melatonin administration at high dose on the photopic and scotopic ERG of beagle dogs.

2. Materials and methods

2.1. Animals

All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the use of animals in ophthalmic and vision research and were approved by our local animal care committee. 7 beagle dogs, 4 males (weight: 9–12 kg, age: 18–20 months) and 3 females (weight: 9–12 kg, age: 18–20 months) were used for this study. Every dog was healthy and had a complete physical examination, including an ophthalmological assessment 72 h prior to the recording of the ERG. Animals were housed individually on a 12-h light/dark cycle with a constant luminance background of 30 cd/m^2 inside the cages.

2.2. ERG recordings

The method for ERG recordings was previously described [28]. Binocular, full field ERGs were recorded in photopic and scotopic conditions from dogs on two occasions: once without melatonin (control condition) and once 60 min following melatonin (Natrol®) administered orally at a dose of 90 mg/dog. All animals were anaesthetized using a single intramuscular injection of a mixture of ketamine (5 mg/kg, Imalgene® 1000, Mérial, France) and medetomidine (0.2 mg/kg, Domitor®, Pfizer, France) 5 min after melatonin ingestion. Corneal hydration was maintained throughout the entire procedure with the use of carbachol (Ocrygel®, TVM, France) applied over the entire cornea. Rectal temperature and heart rate were regularly monitored. All ERGs were recorded between 8:30 and 12:30. The two conditions were tested with an interval of 8 days and at the same time than the previous recording to avoid circadian variability. The flash retinal responses were evoked at 15 different increasing intensities ranging from -3.39 log cd s m^{-2} to 0.81 log cd s m^{-2} (difference of 0.3 log cd s m^{-2} between each intensity) presented first in scotopic condition, following a 32 min dark-adaptation period, and then in photopic condition (luminance background of 30 cd/m^2) following a 16 min light adaptation process. The photopic flicker retinal responses were evoked at 4 different temporal frequencies (6 Hz, 12 Hz, 20 Hz and 30 Hz) delivered for 16 s. Flicker ERG was assessed at the end of the flash photopic ERG recording.

2.3. Data analysis

The ERG response is composed of a negative component called the a-wave, followed by a large positive component, called the b-wave. The a-wave is generated mainly by the photoreceptors, whereas the b-wave originated mostly from the ON-bipolar cells. By convention, a-wave amplitude is measured from the baseline to trough, and the b-wave from the trough of the a-wave to the peak of the b-wave. Implicit times were measured from flash onset. As per Hébert et al. [14], the amplitudes of the b-waves were plotted against flash luminance in order to generate the photopic and scotopic luminance response function (LRF) from which two parameters were derived, namely Vmax, which refers to the saturating b-wave amplitude observed in the luminance response function, and log K which is interpreted as retinal sensitivity and represents the intensity necessary to reach half of the Vmax. Beside the b-wave, a-wave amplitude, a- and b-wave implicit times were also measured.

The reproducibility of the ERG recording method has already been discussed [28]. For each animal, the response obtained from each eye was reported separately. In order to determine if there is any statistical difference between both eyes, a Student paired t-test ($p < 0.05$) was performed for every response obtained between left and right eye. No significant difference was found for any dogs, so the averaged response of both eyes was calculated and reported in Section 3. Repeated-measures analyses of variance (ANOVAs) were performed to assess the effect of treatment in all the ERG parameters (a- and b-waves amplitudes and implicit times) in photopic and scotopic conditions. Analysis of the ERG also included the log K and this variable was analyzed with paired t-tests. All analyses were conducted using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL) ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Standard deviations (SD) of the means are presented in the text with the averaged values of all parameters.

3. Results

Fig. 1 presents a typical example of photopic ERG waveforms obtained before and after melatonin ingestion. At first sight, an important decrease of a-wave and b-wave amplitudes can be observed. The mean photopic LRF for absolute a-wave and b-wave amplitudes are also shown in Fig. 1. A significant group difference was found [$F(1,12) = 57.913$, $p < 0.001$] for the a-wave amplitude and, consequently, the maximal a-wave amplitude was decreased following melatonin ingestion (-49.64 ± 15.69 μV; $p < 0.001$) compared to the control condition (-126.9 ± 16.45 μV). A significant overall difference was also observed before and after melatonin ingestion [$F(1,12) = 59.353$, $p < 0.001$] for the b-wave amplitudes resulting in a Vmax amplitude much diminished after melatonin ingestion (115.40 ± 28.27 μV; $p < 0.001$) when compared to control