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Blue light stimulation of the optic nerve head reduces melatonin levels in rabbit posterior segment

Melatonin is a neurohormone synthesized by the pineal gland and various ocular structures, including the lacrimal gland, corneal epithelium, sclera, iris, ciliary body, lens, and retina. Besides modulating systemic circadian rhythms, melatonin plays an important role in ocular physiological processes such as tear and aqueous humor production, corneal re-epithelialization, or regeneration of photoreceptor outer discs.[1] A specialized set of retinal ganglion cells, known as intrinsically photosensitive retinal ganglion cells (ipRGCs), regulate melatonin synthesis both systemically and within the eye. Stimulation of melanopsin, a photopigment sensitive to short-wavelength light (460–490 nm, blue light) present in the soma, axons, and dendrites of ipRGCs, is responsible for decreasing melatonin levels.[2]

Proper melanopsin and ipRGC function has been demonstrated to be essential for correct refractive development, suggesting a potential therapeutic target for addressing myopia progression.[3] In this regard, recent studies showed that blue light stimulation of ipRGCs resulted in exaggerated axial length responses to short-term hyperopic defocus,[4] as well as changes in choroidal thickness,[5] electroretinogram b-wave amplitude,[6] and dopamine release,[7] which may have a therapeutic role in myopia control. The current study investigated the effects of stimulating ipRGCs on ocular melatonin in rabbits using a surgically inserted optical fiber, contributing to a better understanding of the mechanisms involved in the progression and control of myopia.

A short-term prospective, randomized study was conducted on 15 white New Zealand rabbits. All experimental procedures were carried out as previously described by Carpena-Torres et al.[7] and approved by the Committee on Animal Testing of the Complutense University of Madrid (code: ES280790000086). The rabbits were randomly divided into three groups (n = 5 each) and subjected to blue light (450–500 nm; peak intensity at 470 nm) stimulation of the optic nerve head using an optical fiber for 1 min, 10 min, or no stimulation as a control. In rabbits stimulated for 1 min, the radiant flux of the blue light was 7.0 mW. For rabbits stimulated for 10 min, the radiant flux was increased every 2 min in steps of 1.2, 2.3, 3.5, 4.7, and 5.8 mW. In all rabbits, the left eye was operated on to introduce the optical fiber into the optic nerve excavation, while the contralateral eye served as an internal control. Each rabbit was evaluated on a different day at the same time (10 a.m.) to avoid circadian cycle bias. The surgical procedure, including blue light stimulation, lasted approximately 20–30 min, after which an additional 20 min were waited before euthanizing the animals and collecting biological samples. Melatonin levels in tears, aqueous humor, vitreous body, and retina (including choroid) were measured by HPLC following the protocol described by Alkozi and Pintor.[8] Statistical comparison between melatonin levels of the treated eye and the contralateral eye was performed using the Wilcoxon signed-rank test, with statistical significance established at 95 % ($p < 0.05$). Results are expressed as median [interquartile range; Q1, Q3].

In the control rabbits, which were not stimulated, there were no significant differences ($p \geq 0.05$) in melatonin levels of the vitreous body and retina between the eye operated on to position the optical fiber in the optic nerve head (vitreous body: 0.49 [0.24, 1.54] nM; retina: 0.08 [0.07, 0.31] pmol/μg_{DNA}) and the contralateral eye (vitreous body: 1.16 [0.42, 2.53] nM; retina: 0.14 [0.10, 0.36] pmol/μg_{DNA}).

However, blue light stimulation of the optic nerve head for 1 min significantly decreased (p = 0.043) melatonin concentration in the vitreous body of the stimulated eye (0.37 [0.25, 0.79] nM) compared to the contralateral eye (1.02 [0.37, 1.87] nM). Additionally, stimulation for 10 min significantly reduced (p = 0.043) melatonin levels in both the vitreous body and retina of the stimulated eye (vitreous body: 0.31 [0.19, 0.44] nM; retina: 0.20 [0.09, 0.31] pmol/μg_{DNA}) compared to the contralateral eye (vitreous body: 0.46 [0.41, 1.30] nM; retina: 0.32 [0.19, 0.48] pmol/μg_{DNA}). The HPLC system lacked the sensitivity to detect melatonin signals in tears and aqueous humor.

These results provide the first *in vivo* evidence that selective blue light stimulation of melanopsin expressed in the ipRGCs is presumably responsible for reducing melatonin levels in the posterior segment of the eye. This biochemical response would also be accompanied by an increase in dopamine concentration in the aqueous humor and vitreous body, as Carpena-Torres et al.[7] found in previous experiments using the same methodology in rabbits. Nevertheless, the lack of control groups with mid- (green) and long-wavelength (red) light stimulation in this experiment prevented understanding whether ipRGCs exclusively mediate melatonin synthesis or if other signaling pathways involving additional photoreceptors might exist.[9]

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Despite the specific function that melatonin plays in ocular axial growth remaining unknown, it is known that dopamine signaling is directly involved through D_1 and D_2 membrane receptors.[10] Since dopamine released after blue light stimulation potentially has a therapeutic role in controlling myopia progression, it was hypothesized that melatonin signaling through MT_1 or MT_2 receptors located in photoreceptors, inner retinal neurons, RGCs, and even the sclera might also have such a role, aside from triggering dopamine release.[9] Recent studies conducted in humans, where ipRGCs were selectively stimulated with blue light, support the idea that melatonin, through this mechanism, could regulate physiological processes involved in refractive development such as axial growth or choroidal blood flow.[4-6]

Finally, it should be noted that melatonin, besides having antioxidant properties, regulates numerous ocular processes in both the anterior and posterior segments of the eye. Thus, reducing its levels with blue light may negatively impact other ocular conditions such as dry eye, corneal wound healing, cataract formation, and retinal diseases like glaucoma, age-related macular degeneration, or diabetic retinopathy.[1]

Declaration of competing interest

The authors have no conflicts of interest to declare.

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