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Removal of melatonin receptor type 1 increases intraocular pressure and retinal ganglion cells death in the mouse

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ABSTRACT

Previous studies have demonstrated that melatonin is effective in lowering intraocular pressure and that it may also protect ganglion cells. We have recently reported that, in mice lacking the melatonin receptors type 1, 25–30% ganglion cells die out by 18 months of age, suggesting that these receptors might be important for ganglion cells survival. In this study we show that the loss of ganglion cells is specific for melatonin receptors type 1 knock-out since mice lacking the melatonin receptors type 2 did not show any significant change in the number ganglion cells during aging. Furthermore, we report that melatonin receptors type 1 knock-out mice have higher intraocular pressure during the nocturnal hours than control or melatonin receptors type 2 knock-out mice at 3 and 12 months of age. Finally, our data indicate that administration of exogenous melatonin in wild-type, but not in melatonin receptors type 1 knock-out, can significantly reduce intraocular pressure. Our studies indicate that the decreased viability of ganglion cells observed in melatonin receptors type 1 knock-out mice may be a consequence of the increases in the nocturnal intraocular pressure thus suggesting that intraocular pressure levels at night and melatonin signaling should be considered as risk factor in the pathogenesis of glaucoma.

Introduction

Previous studies have shown that melatonin modulates many important functions within the eye [2,19]. Melatonin exerts its influence by interacting with a family of G-protein-coupled receptors that are negatively coupled with adenylate cyclase [8]. Melatonin receptors belonging to the subtypes MT_1 and MT_2 have been identified in the mammalian retina. MT_1 receptors are found in the inner nuclear layer (horizontal and amacrine cells), in the inner plexiform layer, in retinal ganglion cells (GCs) and in the retinal pigmented epithelium of the rat retina [7]. In humans, melatonin receptors have been located on the rod photoreceptors and on GCs [11]. In the mouse, MT_1 mRNAs have been localized to photoreceptors, inner retinal neurons and GCs [5]. Previous studies have also reported that melatonin receptors are present in the ciliary body [3,12,13,20]. The expression of melatonin receptors in the iris and ciliary body processes has led to the hypothesis that melatonin may be involved in the regulation of IOP and indeed several studies have shown that melatonin can modulate the IOP in several species [13–16].

However, it is worth noting that a clear dissection of the role played by melatonin and specific melatonin receptors in the mammalian eye is not well defined. This lack of data is due to the fact that the vast majority of mouse strains are genetically incapable of synthesizing melatonin in the pineal and/or retina [17] so very few studies have compared retinal physiology in melatonin-proficient and melatonin-deficient mice. Our laboratory has recently produced mice with targeted deletion of the MT_1 or MT_2 receptor gene in a melatonin proficient background (C3H/f+/+). These mice are capable of synthesizing melatonin, lack the MT_1 or MT_2 receptors, and do not develop retinal degeneration [5]. We have recently reported that removal of the MT_1 receptor leads to a significant loss (25–30%) in the number of cells within the retinal ganglion cell layer (RGL) during aging [5]. Since elevated IOP is considered to be one of the most important factors that may lead to GCs loss [9] we decided to investigate the role of melatonin and melatonin receptors on the regulation of IOP.

C3H $MT_1^{-/-}MT_2^{-\backslash-}$ knock-out mice homozygous for the *rd1* mutation, generously donated by Drs. Reppert and Weaver (University of Massachusetts Medical School), were back-crossed with C3H/$f^{+/+}$ mice in which the *rd1* mutation has been removed to produce C3H/$f^{+/+}MT1^{-/-}$ ($MT_1^{-\backslash-}$) and C3H/$f^{+/+}MT_2^{-/-}$ ($MT_2^{-\backslash-}$) as previously reported in [5]. Mice were maintained in 12 Light:12 Dark (LD) conditions (light on at 06:00 am and off at 06:00 pm) with food and water ad libitum.

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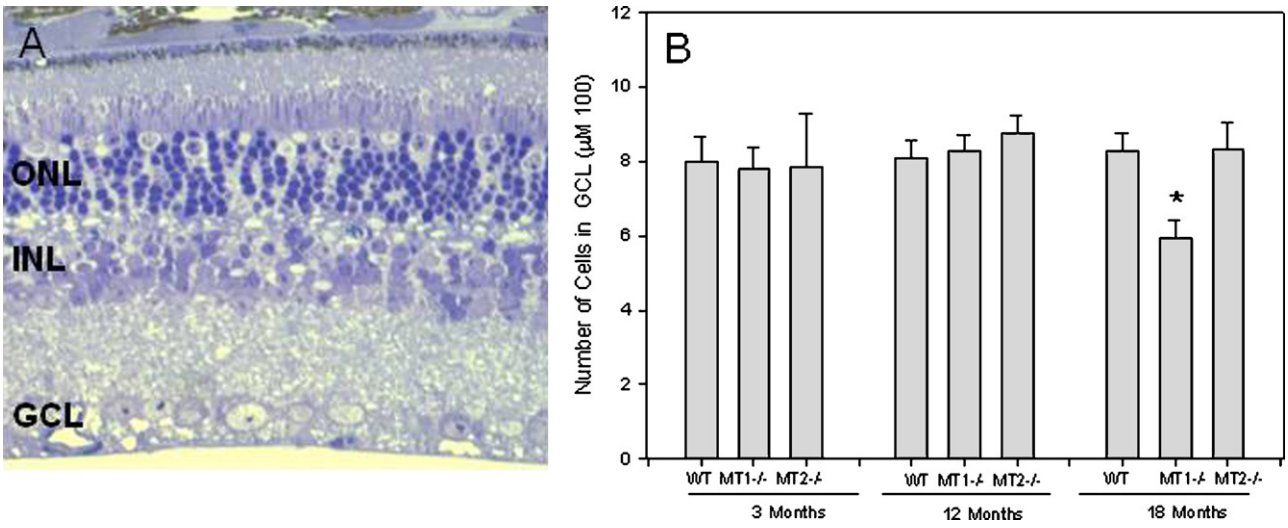

Fig. 1. (A) Photomicrograph of the superior central retinal of $MT_2^{-/-}$ at the 18 months of age. Although a clear reduction in the number of cell nuclei is present in the outer nuclear layer (ONL) no changes are detectable in the inner nuclear layer (INL) or in the ganglion cell layer (GCL). (B) Number of cells in the RGL in the central superior retina of WT, $MT_1^{-/-}$ and $MT_2^{-/-}$ mice at different ages. A significant difference in the number of cells in the RGL is present between WT and $MT_1^{-/-}$ and $MT_1^{-/-}$ and $MT_2^{-/-}$ at 18 months of age. Each bar represents the mean ± SEM; $n = 4$–6. $^*P < 0.05$ (One-Way ANOVA followed by Holm–Sidak tests). Data for the WT and $MT_1^{-/-}$ have been redrawn from [4].

To determine the number of cells in the RGL mice were euthanized and before eye nucleation the superior cornea was marked with a hot needle. After a 1-h fixation in 4% paraformaldehyde, the anterior segment was removed, except for the superior cornea, and the eyes were fixed overnight at 4 °C. After dehydration through a graded ethanol series, eyecups were embedded in Durcopan and sectioned (1.5 μm thickness) and stained with toluidine blue. For each sample the number of cells in the RGL was counted in a 100 μm microscopic field in 10 different locations within each of three adjacent sections. The cells were counted using the Image-Pro Plus 3.0 software. The data obtained from the different adjacent sections were combined and the mean ± standard error of the mean (SEM) was calculated. Comparisons among the different genotypes were carried out using parametric ANOVA followed by a multiple comparison test, where appropriate. Morphometric measurements were made by observers who were blinded to the genotype and age of the samples.

To measure the IOP mice were briefly anesthetized with 2% isoflurane and the IOP was measured using a TonoLab-Tonometer (Icare Finland Ltd.). IOP was measured in each mouse at 3 h interval during the course of the day. The IOP was measured 10 times in each mouse and time point 10 times and an average was then calculated. IOP data are expressed as the mean ± SEM. IOP measurements at Zeitgeber Time (ZT) 15, 18 and 21 (i.e., during the night) were performed under red dim light (<1 lux) to minimize any interference to the dark condition. To measure the effect of exogenous melatonin administration on IOP mice were briefly anesthetized and the IOP was measured as previously described and then were injected intra-peritoneally (i.p.) with 1 mg/kg of melatonin (Sigma–Aldrich, St. Louis, MO). The IOP was then measured again after 1 and 2 h. This dose was selected because it has been shown that it is capable of inducing physiological levels of melatonin in the eye [6] and can affect retinal function [5]. In order to avoid possible complications due to receptor desensitization, mice were injected at ZT12 (i.e., when IOP is already elevated but the endogenous melatonin levels are still low). Control animals were treated exactly as the experimental ones with the only exception that they received an injection of vehicle. Comparisons of IOPs at each time point between groups were also performed by the ANOVA followed by Holm–Sidak tests. $P < 0.05$ was considered statistically significant. All procedures and protocols (number 10-07) were approved by the Morehouse School of Medicine Institutional Care and Use Committee.

In Fig. 1A is shown a photomicrograph of the central superior retina obtained from $MT_2^{-/-}$ mice at 18 months of age. Although $MT_2^{-/-}$ showed a reduction in the number of cells in the photoreceptor layer similar to that observed in $MT_1^{-/-}$ the number of cells in RGL did not show changes during aging in WT and $MT_2^{-\backslash -}$. A significant change in the number of cells was observed between WT and $MT_1^{-/-}$ and between $MT_1^{-/-}$ and $MT_2^{-/-}$ at 18 months of age (One-Way ANOVA followed by Holm–Sidak tests, $P < 0.05$).

IOPs measured under LD conditions showed a significant 24-h rhythm in all three genotypes and at the three ages (One-Way ANOVA, $P < 0.05$) and the IOPs were low during the light phase and high during the dark phase (Fig. 2A–C). When IOPs measured at each time point were compared between WT, $MT_1^{-/-}$ and $MT_2^{-/-}$ mice, the IOPs of $MT1^{-/-}$ mice at three months of age were significantly higher than those measured in age-matched WT or $MT_2^{-/-}$ at ZT 18 and ZT 21 (Fig. 2A). The same pattern was present in 12 months old mice, IOPs at night (ZT 12-18) were significantly higher in $MT_1^{-/-}$ than those recorded in WT and $MT2^{-/-}$ (Fig. 2B, One-Way ANOVA followed by Holm–Sidak test, $P < 0.05$). WT and $MT_2^{-/-}$ mice at 18 months also showed a significant increase in IOP at night, although the increase in aged WT and $MT_2^{-/-}$ was less pronounced that in $MT_1^{-/-}$ and not significant difference was observed among the three genotypes (Fig. 2C, One-Way ANOVA, $P > 0.05$ in all cases). No differences among the three genotypes were observed in the IOPs during the light phase (One-Way ANOVA, $P > 0.05$ in all cases).

As shown in Fig. 3, melatonin significantly lowered IOP levels (One-Way ANOVA followed by Holm–Sidak test, $P < 0.05$) in WT mice when injected at ZT12, but it did not have any effect when injected at in WT at ZT6 (t-test, $P > 0.1$) or in $MT_1^{-/-}$ or $MT_2^{-/-}$ mice (One-Way ANOVA, $P > 0.1$ in all cases).

In a previous investigation we reported that $MT_1^{-/-}$ mice show a significant reduction in the number of cell in the RGL during aging with respect to the number observed in aged matched WT mice [5]. In this study, we report that genetic removal of the MT_2 receptors does not affects the viability of cells in RGL during aging (Fig. 1) thus suggesting that the lost of cells in this retinal layer is specific for MT_1 receptors. Our data also indicate that the changes in the number of cell in the RGL observed in $MT_1^{-/-}$ is not a consequence of pho-