MT₁ Melatonin Receptor in the Human Retina: Expression and Localization

Judite Scher,¹ Ellen Wankiewicz,¹ Gregory M. Brown,¹ and Hiroki Fujieda¹,²

PURPOSE. Melatonin’s function in human vision is far from understood, in part because of the lack of information on its cellular targets. Therefore, expression and localization of the MT₁ melatonin receptor in human retina was examined.

METHODS. Postmortem nonpathologic human eyes from nine donors were investigated, three by reverse transcription–polymerase chain reaction (RT-PCR) for MT₁ and MT₂ transcripts and six by immunocytochemistry, using a peptide-specific anti-MT₁ receptor antibody.

RESULTS. RT-PCR suggested that both MT₁ and MT₂ transcripts had similar levels of expression. Vertical slices of human retina demonstrated MT₁ immunoreactivity in cell bodies along the outer border of the inner nuclear layer (INL), along the inner border of the INL, in cell bodies within the ganglion cell layer (GCL), and in the inner segments (IS) of photoreceptors. Double immunolabeling with anti-parvalbumin, a horizontal cell marker, showed that MT₁-positive cells along the outer INL border were exclusively horizontal cells, and that 18% of horizontal cells in central retina expressed MT₁. Double staining with MT₁ and markers for both rod and cone photoreceptors suggest that IS staining is present on rod cells.

CONCLUSIONS. The MT₁ receptor is expressed in diverse neuronal cell types in the human retina, providing evidence of a significant role for melatonin and this receptor subtype in human vision. (Invest Ophthalmol Vis Sci. 2002;43:889–897)

Melatonin is produced in the retina by photoreceptor cells in most vertebrate species studied to date,¹,² including humans.³ Synthesized and released predominantly at night,⁴ melatonin regulates physiological functions implicated in retinal adaptations to low light intensities, including dark-adaptive cone elongation in Xenopus,⁶ activation of rod photoreceptor disc shedding in Xenopus and rat,⁷,⁸ enhancement of horizontal cell sensitivity in salamander,⁹ and horizontal cell dark adaptations in fish.¹⁰

Melatonin appears to have an antagonistic interaction with dopamine,¹¹,¹² which, produced during the day, has been established as the major light-adaptive signal within the vertebrate retina.¹² Melatonin inhibits the calcium-dependent release of dopamine in rabbit,¹³ chick,¹⁴ and Xenopus retinas.¹⁵ In contrast, dopamine inhibits melatonin biosynthesis in hamster,¹⁶ Xenopus,¹⁷ and chick retinas.¹⁸ This mutual inhibition has been suggested to be the mechanism responsible for retinal adaptation to changes in light intensities. However, most information available on the function of retinal melatonin has been acquired from lower vertebrates, who, unlike mammals, have a fixed pupillary aperture and thus may invoke a unique method of retinal adaptation to changes in light intensity. Evidence for melatonin’s function or its cellular targets in mammalian retina, specifically in humans, is limited, leaving its role in human vision undefined.

Melatonin is believed to elicit its biological effects through a distinct family of G-protein–coupled receptors.¹⁹,²⁰ In a previous study, two melatonin receptor subtypes, the MT₁ and MT₂ receptors (formerly Mel₁a and Mel₁b, respectively,²¹), were identified in human retina by RT-PCR,²² demonstrating higher levels of MT₁ expression. High-affinity 2-[¹²⁵I]-iodomelatonin–binding sites have also been identified in the inner plexiform layer (IPL) of rabbit, mouse,²³ and chicken.²⁴ We have recently reported MT₁ receptor mRNA expression in ganglion, amacrine, and horizontal cells and have localized MT₁ protein in the outer plexiform layer (OPL) and IPL in rat retina²⁵ and in dopaminergic and γ-aminobutyric acid (GABA)ergic amacrine cells in guinea pig retina.²⁶ However, species differences in melatonin binding and receptor expression are well established in both brain²⁷ and retina.²⁸,²⁹,³⁰ Therefore, determining receptor expression in human retina is crucial to understanding melatonin’s role in human vision.

Melatonin’s effects in humans have most often been examined in biological rhythms,³⁰ sleep disorders,³¹ and mental illness.³² Although established as an important neuromodulator in vertebrate retina, virtually nothing is known of melatonin’s role in human vision. Therefore, we investigated the cellular targets of melatonin in human retina and herein report the immunocytochemical localization of the MT₁ receptor in rod, horizontal, dopaminergic amacrine, and ganglion cells. Furthermore, RT-PCR shows similar expression levels of MT₁ and MT₂ in human retina, suggesting a significant role for both receptor subtypes in human vision.

METHODS

Human Retinal Tissue Preparation

Approval for the use of human tissue was provided by the Ethics Review Committee at the Office of Research Services, University of Toronto (Protocol Number 5940), and the protocol is in adherence to the Declaration of Helsinki for research involving human subjects. Nine human eye shells (enucleated whole globes without cornea) were provided by the Eye Bank of Canada, Ontario Division, with donor information and consent for research (Table 1). Time between death and enucleation was under 12 hours, and time between death and fixation was under 24 hours in all samples. Both time between death and enucleation and time between death and fixation are provided, because a previous report has suggested that variability in morphologic preservation may be influenced by both of these factors.³³ All samples were found to be negative for HIV and hepatitis B and C. For immu-
nocytochemistry, whole eye cups were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 6 hours, rinsed in 30% sucrose in PBS, frozen with dry ice-acetone, sliced to 10-μm-thick sections on a cryostat, and collected on frosted glass slides (Superfrost Plus; Fisher Scientific, Fairlawn, NJ). For RT-PCR, neural retina was dissected, frozen on dry ice and stored at −70°C until use. All samples used in this study were free of malignancies or any known ocular disease.

Reverse Transcription–Polymerase Chain Reaction

RT-PCR was conducted as described previously on three separate samples. Briefly, total RNA was extracted from the retina using the acid-phenol method and treated with DNase (Roche Diagnostics, Mannheim, Germany) to avoid contamination by residual DNA. First-strand cDNA was synthesized using a First-Strand Synthesis System (Superscript; Life Technologies, Gaithersburg, MD). The cDNA reaction product (2 μL) was subjected to 34 to 40 cycles of PCR amplification in a reaction volume of 25 μL containing 1× PCR buffer, 0.025 U/μL Taq DNA polymerase (Life Technologies), and 0.2 mM dNTP mix, and the human MT1- and MT2-specific oligo primers were added. Primer design was based on those previously reported by Reppert et al. The MT1 primers were 5′-TCTCCTGTGATCCTCTC-CACT-3′ and 5′-CTGTGATACGTGTTGACTG-3′, which amplified a cDNA fragment of 286 bp. Those for MT2 were 5′-TCTCCTGTGATCCTCTC-GTGCTCA-3′ and 5′-AGCCAGATGGAGCATGTGCAGA-3′, to amplify a fragment of 322 bp. Each reaction cycle consisted of: 1 minute at 94°C, 1 minute at 65°C, and 1 minute at 72°C. Control reactions were performed using the PCR mixture without the cDNA template or using RNA samples without RT. The identities of PCR products were further verified by restriction analysis. BstXI restriction enzyme (Fermentas, Burlington, Ontario, Canada) was used to generate 115- and 171-bp fragments from the MT1 cDNA product, and Apal (Fermentas) was used to digest MT2 cDNA products into 144- and 178-bp fragments. The sizes of the amplified DNA fragments were confirmed using 2% agarose gel electrophoresis.

Antibodies

A polyclonal anti-MT1 receptor antibody directed against a peptide sequence corresponding to the third intracellular loop of the human MT1 receptor (residues 226-238; KPKLKPQDFRNFV) was affinity purified as previously described, and used in the present study. This antibody is well characterized and has been shown to specifically label the melatonin MT1 receptor in rat25 and guinea pig36 retinas, hypothalamus of human and rat, cerebellum of human, guinea pig kidney, and small intestine, as well as HEK-295 cells. The possibility of cross-reactivity of this antibody with the MT2 receptor was tested by preincubation of the MT1 antibody with a peptide corresponding to the same region of the human MT2 receptor (RCLKPSDLRSFL). The MT2 peptide did not block the signal obtained by immunostaining, further indicating specificity of this antibody to the MT1 receptor (data not shown).

Immunocytochemistry

MT1 immunostaining was performed by the streptavidin-biotin method and by signal amplification using the TSA Biotin System (NEL Life Science, Boston, MA). Briefly, sections were treated with 0.3% hydrogen peroxide in methanol for 20 minutes and sequentially incubated with blocking reagent. Cy3 signal was completely eliminated by the omission of the anti-MT1 antibody or preabsorption with its target peptide, and the FITC signal was undetectable by double immunolabeling was performed using monoclonal antibodies to either TH diluted to 1:1500, Parv to 1:8000, Calb to 1:500, or Opsin to 1:3, as described. Sections were sequentially incubated in a mixture of primary antibodies overnight, a mixture of FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) secondary antibody diluted to 1:1,000, in combination with anti-MT1. All dilutions were performed in 0.1 M PBS (pH 7.4) containing 0.05% Triton X-100 and 1% bovine serum albumin, except for biotin tyramide which was diluted with the manufacturer-provided diluent (NEL). All monoclonal antibodies were labeled with fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse IgG (Jackson ImmunoResearch) secondary antibody diluted to 1:1,000, whereas the anti-MT1 receptor antibody was visualized with Cy3, as described. Sections were sequentially incubated in a mixture of primary antibodies overnight, a mixture of FITC-conjugated goat anti-mouse IgG and biotinylated swine anti-rabbit immunoglobulins 1:2,000 (Dako, Glostrup, Denmark) for 30 minutes, peroxidase-conjugated streptavidin 1:2,000 (Dako) for 30 minutes, biotin-conjugated tyramide 1:50 (NEN) for 8 minutes, and, finally, Cy3-conjugated streptavidin 1:800 (Jackson ImmunoResearch, West Grove, PA) for 30 minutes. Immunocytochemical controls were performed by using primary antibody preabsorbed with an excess amount of immunogen peptide or by omission of primary antibody. Double immunolabeling was performed using monoclonal antibodies to either TH diluted to 1:1,500, Parv to 1:500, or Opsin to 1:10,000, in combination with anti-MT1. All dilutions were performed in 0.1 M PBS (pH 7.4) containing 0.05% Triton X-100 and 1% bovine serum albumin, except for biotin tyramide which was diluted with the manufacturer-provided diluent (NEL). All monoclonal antibodies were labeled with fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse IgG (Jackson ImmunoResearch) secondary antibody diluted to 1:1,000, whereas the anti-MT1 receptor antibody was visualized with Cy3, as described. Sections were sequentially incubated in a mixture of primary antibodies overnight, a mixture of FITC-conjugated goat anti-mouse IgG and biotinylated swine anti-rabbit immunoglobulins for 30 minutes, peroxidase-conjugated streptavidin for 30 minutes, biotin-tyramide for 8 minutes, and, finally with Cy3-conjugated streptavidin for 30 minutes. Cross-reactivity of secondary antibodies was tested by omitting one of the primary antibodies. Cy3 signal was completely eliminated by the omission of the anti-MT1 antibody or preabsorption with its target peptide, and the FITC signal was undetectable by

### Table 1. Human Donor Information

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Time between death and fixation = time between death and enucleation + time between enucleation and fixation. MT1 expression was found to be independent of time, and cause of death, and postmortem delay, both by RT-PCR analysis and patterns of immunocytochemistry. MI, myocardial infarction; MVA, motor vehicle accident; CVA, cerebral vascular accident.
Quantitation of MT1 expression in dopaminergic amacrine cells was performed across the entire length of human retina, because of their very low density. However, because of the large number of horizontal cells, quantitation of MT1 expression in horizontal cells was confined to the central retina, defined as 2 mm of inner nuclear layer (INL) from the foveal border. Colocalization is expressed as percentage of cells positive for both antibodies ± SEM. A minimum of eight sections for each sample in three separate experiments were analyzed, and the means determined by one-way ANOVA. Measurements of retinal length were performed on a laser scanning confocal microscope (Zeiss) equipped with an imaging system (LSM410; Zeiss). Statistical analysis of the data herein was examined using statistical software (SPSS Science, Chicago, IL). Significance was evaluated at P < 0.05.

Degradation by Postmortem Delay
To determine the impact of postmortem delay in fixation on the detection of MT1 immunoreactivity in human retinal samples, a postmortem degradation study was undertaken in an animal model, adapted from the protocol of Roufai and Rees.26 Because we have previously reported that many ganglion cells in the guinea pig retina are immunoreactive to the MT1 antibody25 the degeneration in MT1 immunoreactivity in this cell type, with increased time between death and tissue fixation was examined. Because anti-TH signal degradation with increased postmortem delay has been previously reported in primates,25 postmortem degradation in TH immunoreactivity in the guinea pig retina19,26 was performed as a control. Ten, 22-day-old Hartley guinea pigs kept under an artificial light–dark cycle (LD 12:12) with lights on at 8 AM were killed during the photo phase between 12 and 3 PM and whole eyes dissected. Four control eyes were immediately fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) by immersion for 30 minutes at 4°C and prepared as outlined earlier. Conditions simulating those experienced by human eye samples after enucleation were reproduced so that the remaining eyes were kept at 4°C for 6, 12, 18, and 24-hour time intervals between enucleation and fixation in a moist chamber. A minimum of three sections from three to four animals within each time point of fixation after death were stained with anti-MT1 at a concentration of 1:150 or anti-TH antibodies at 1:10,000, as outlined earlier. The number of MT1 immunoreactive ganglion cells in a 3-mm length of ganglion cell layer (GCL) from the ora serrata inward, and the number of CA1 and CA2 amacrine cells in 10- to 20-mm lengths of INL from the ora serrata were counted. Variance in the means from each fixation time point was assessed by one-way ANOVA using statistical analysis software (SPSS) with significance set at P < 0.05. The number of immunoreactive cells per millimeter per time point was compared by a post hoc Tukey honestly significant difference (HSD) test. The experimental procedures used in this study were in accordance with the guidelines of the Canadian Council on Animal Care and a research protocol approved by the Centre for Addiction and Mental Health Animal Care Committee, Clarke Institute of Psychiatry Division, and adhere to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RESULTS
mRNA Expression of MT1 and MT2 Melatonin Receptor Subtypes in the Human Retina
RT-PCR analysis of human retina demonstrated that both MT1 and MT2 melatonin receptor transcripts were present and could be identified as bands with expected sizes of 286 and 322 bp, respectively, (Fig. 1). Although quantitation was not performed, it appears that similar levels of expression of MT1 and MT2 mRNA were detected in three separate samples tested. Restriction analysis verified specific amplification of both receptor subtypes. No detectable amplification was observed using control samples.

Localization of the MT1 Receptor in Human Retina
Vertical slices of human retina immunohistochemically stained with the anti-MT1 antibody showed specific immunoreactivity in a number of cell types. Figures 2A–D display a typical example of human retina within the foveal border region (at an extremity of approximately 1.5 mm from the foveal border). Expression of the receptor was identified in cell bodies along the outer border of the INL, most of them immediately adjacent to the OPL (Figs. 2B, 2C). Because of positioning and morphology, these cells were presumed to be horizontal cells. Positive staining was also identified in cells lining the inner boundary of the INL, which were thought to be amacrine cells, as well as numerous cell bodies in the GCL, most of which were assumed to be ganglion cells because of their large somal size. Control slides subjected to the anti-MT1 antibody after preabsorption
with target peptide showed a marked reduction in specific signal (Fig. 2D), except for faint nonspecific signal present in the IS of photoreceptors morphologically similar to cone cells (Fig. 2D, arrowheads), suggesting specificity of the MT1 antibody. A number of displaced cell bodies in the IPL were also MT1 positive and may represent displaced amacrine or ganglion cells (Fig. 2C, double arrow). Strong MT1 immunoreactivity was also detected in the IS of a subset of photoreceptors (Fig. 2E, arrows), pictured at an eccentricity of approximately 5 mm from the foveal border. This staining was present throughout most of the retina but was not identified in either the fovea (Fig. 2B) or ora serrata (not shown). This intense labeling was completely blocked by preincubation of the MT1 antibody with target peptide (Fig. 2F), again indicating the specific nature of immunoreactivity obtained. The pictured images are from human retinal sample 6, but are representative of the staining pattern observed in all samples examined.

Expression of the MT1 Receptor in Dopaminergic Amacrine Cells

Because the mammalian retina is believed to contain as many as 50 subtypes of amacrine cells, each with varying function and neurochemical composition,50 determining the subtypes of amacrine cells that express the MT1 receptor protein is essential for understanding melatonin’s role in the retina. Because of the closely related interactions between melatonin and dopamine in the retina, dopaminergic neurons were examined for expression of melatonin receptors. Two types of dopaminergic cells were discriminated in the human retina by location, shape, and amount of immunolabeling with the anti-TH antibody. CA1 dopaminergic amacrine cells were identified along the vitreal border of the INL. These cells displayed intense immunoreactivity to TH, had a rounded or elongated cell body, and were present in very low density (Fig. 3A).35,51 Of the CA1 cells, 69.2% were found to colocalize with the MT1 receptor (Fig. 3B). A second type of TH-immunoreactive cell, the CA2, also showed a low density distribution, possessed irregular or elongated cell bodies, but displayed much weaker TH immunoreactivity and was usually located along the innermost border of the INL, but was also found in the IPL or GCL (Fig. 3C).35,36 A population of these cells again colocalized with the MT1 antibody, in that 63.2% showed MT1 expression (Fig. 3D).

Expression of the MT1 Receptor in Horizontal Cells

To determine whether MT1 immunoreactive cell bodies along the outer INL border were horizontal cells, double immunola-

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Expression of MT$_1$ in Rod Photoreceptor Inner Segments

To determine the type of photoreceptor that expresses the MT$_1$ receptor antibody, human retinal samples were subjected to antibodies of Calb, a cone photoreceptor marker, as well as those of opsins, a rod marker. Staining of human sections with Calb showed immunoreactivity in cone IS and cell bodies, as reported previously (Fig. 5B, arrows). In double immunolabeling experiments, strong MT$_1$ staining was observed in the IS of photoreceptors (Fig. 5A, arrowheads) that were not labeled with Calb (Fig. 5B, arrowheads), suggesting that the MT$_1$ receptor signal was present on rod photoreceptor IS. Weak MT$_1$ signal was also identified in cone photoreceptors (Fig. 5A, arrows) that were also strongly reactive to Calb (Fig. 5B, arrows). However, as was shown previously (Figs. 2D, 2F) this staining was not blockable by preincubation with target peptide and could not be considered specific. Double immunostaining of human retina with Opsin and anti-MT$_1$ demonstrated MT$_1$ immunoreactivity in the IS of photoreceptors (Fig. 5C, arrowheads) and Opsin staining in the outer segments (OS) of rod photoreceptors, as reported$^{46-47}$ (Fig. 5D, arrows). Figure 5E is an overlay of the images in Figures 5C and 5D, clearly demonstrating the colocalization of the Opsin and MT$_1$ antibodies in the same photoreceptor cell population, suggesting that MT$_1$ is expressed in rod photoreceptor IS.

Effects of Postmortem Delay on MT$_1$ and TH Immunoreactivity

Because there was a postmortem delay in the fixation of the human tissue used herein, an adjunct control study was conducted in guinea pig retina to assess the effect of increasing delay between death and fixation on MT$_1$ and TH immunoreactivity. A significant decrease in both anti-MT$_1$ and anti-TH immunoreactivity was detected after a 12-hour postmortem delay (Fig. 6A, 6B, asterisk), which is in accordance with the previous report on TH degradation in primate retina.$^{36}$ Compared with a 0-hour postmortem delay in fixation, the level of immunodetection did not degrade further between 12 and 24 hours in both MT$_1$-stained ganglion cells and TH-stained CA1 cells, remaining stable between 45% to 60% detection. A further decline in immunodetection was observed in CA2 cells between the 12- and 18-hour postmortem period (Fig. 6B, double asterisk), which may reflect the lower levels of TH inherent in CA2 neurons.
DISCUSSION

The focus of this investigation was to determine the cell types directly targeted by melatonin in the human retina through the MT₁ receptor. Because species differences in melatonin receptor expression have been reported both in brain and retina, determining MT₁ expression patterns in the human is essential for understanding melatonin’s role in human vision.

An adjunct to this study examined the impact of postmortem delay on MT₁ immunoreactivity in an effort to exclude this variable from the interpretation of our data. We were able to identify a significant decline in immunoreactivity with a delay of at least 12 hours, and immunoreactivity was stable between 12 and 24 hours. Because seven of the nine human samples examined in the study were subjected to a postmortem delay in fixation within this period, expression and colocalization of.
the MT₁ receptor may be significantly underestimated, suggesting a far greater level of expression for MT₁ than reported herein. However, variations in immunoreactivity caused by distinct postmortem delay may be negligible between samples collected between 12 and 24 hours after death. In fact, we did not find significant differences in levels of MT₁ expression between samples. Despite the reduction in the number of immunoreactive MT₁ and TH neurons, there was no change in distribution or cell morphology in either the guinea pig or the human samples examined. Furthermore, the pattern and intensity of MT₁ expression appeared similar in all samples examined, independent of time or cause of death. Further quantitation may be necessary to determine whether MT₁ protein expression shows a circadian rhythmicity in the human retina, as reported in melatonin receptor mRNA expression in the *Xenopus* retina.⁵³ ⁵⁴

We report similar levels of expression of both the MT₁ and MT₂ melatonin receptor subtypes in the human retina. A previous report identifying both melatonin receptor transcripts in human retina by RT-PCR suggests that the MT₂ receptor shows a considerably higher level of expression and may thus be the major melatonin receptor subtype in this tissue. Because commercially obtained poly(A)-RNA had been used, as opposed to fresh tissue as reported herein, the discrepancy in results may arise from the diverse origins of mRNA. The three specimens examined in the current study by RT-PCR analysis were all postmortem samples with no ocular disease, and despite being harvested at different times of day, showed similar results, suggesting reproducibility of these data. Because the primers used in both reports were identical, this could not account for the disparity in results. However, receptor expression levels were not quantitated, either in this study or the previous report, so that critical analysis using real-time PCR or competitive RT-PCR must be undertaken, before determining the relative expression levels of both melatonin receptor subtypes in the human retina.

The present study is the first to localize MT₁ expression to horizontal, amacrine, and ganglion cell bodies as well as inner segments of rod photoreceptor cells in the human retina by immunocytochemistry. Melatonin receptor expression in dendritic processes, identified in our previous immunocytochemical work as well as in 2-iodomelatonin-binding studies in rabbit and chick, were absent in human, perhaps because of postmortem degradation of protein expression. Localization of the MT₁ receptor in horizontal, amacrine, and ganglion cells are common in human and rodent retina, suggesting a conserved function of MT₁ in these mammalian species.

This study is also the first to establish expression of MT₁ in most dopaminergic amacrine cells in human retina. Substantial evidence suggests that melatonin inhibits the stimulation-evoked release of dopamine in the retinas of *Xenopus*, fish, rabbit, and chick. Through a specific receptor-mediated mechanism. Although there is no evidence suggesting the same is true in humans, the present finding of melatonin receptors on 69% of CA1 and 63% of CA2 dopaminergic cells in human retina implies that melatonin may modulate dopaminergic function by directly acting on these populations through the MT₁ receptor. In guinea pig retina, it was found that 100% of CA1 and 40% of CA2 cells express MT₁. This interspecies discrepancy in MT₁ expression by dopaminergic neurons cannot be explained by this study. However, guinea pig retina immersion fixed, rather than perfused, showed a decreased population of CA1 cells stained with the MT₁ antibody (data not shown), suggesting fixation methods may influence detection of MT₁ and TH colocalization. That postmortem degradation of MT₁ immunoreaction in human retina may result in an underestimation of MT₁ expression in TH-positive cells cannot be excluded. It has recently been reported that inhibition of dopamine by melatonin is mediated through the MT₂ receptor in rabbit retina.⁶⁰ Because this work has not been repeated in any other species, it is unknown whether MT₂ plays a similar role in other species, or whether species differences in melatonin receptor subtype function may exist in this neural population. Alternatively, both receptor subtypes may be expressed on the dopaminergic cells, but may perform unique functions, suggesting an as yet unknown function for the MT₁ receptor in dopamine regulation. However, the finding of MT₁ receptor expression on most dopaminergic amacrine cells in both human and guinea pig points to a significant function of MT₁ in this amacrine cell subtype. Further investigation is needed to determine the relative roles of MT₁ and MT₂ on the mammalian retina, in dopamine regulation specifically, as well as light adaptation in general. Because the dopaminergic population represents approximately 1% of all MT₁-positive amacrine cells, most of the MT₁-expressing amacrine cells remain to be characterized.

This report is also the first to identify horizontal cells as direct targets for melatonin in human retina. Horizontal cells provide feedback inhibition to photoreceptors and mediate surround responses through extensive gap junction coupling.⁶³ Dopamine has been shown to decrease horizontal cell gap junction permeability in fish, rat, and rabbit and more recently, in mouse retina, presumably mediated through the D₁ dopamine receptor.⁶⁴ Therefore, it is postulated that dopamine mediates the light-adaptive uncoupling of horizontal cells, but to date, the agent mediating the dark-adaptive coupling has not been identified. Melatonin has been shown to enhance horizontal cell sensitivity in salamander and more recently, in fish retina, it has been shown to act as a potent signal for horizontal cell adaptation to darkness, altering cell morphology by causing breakdown of synaptic spines, which are known to form in response to dopamine administration. The finding that MT₁ receptors are expressed on horizontal cells in human retina and previously in rat⁶⁵ may suggest a further interaction between dopamine and melatonin in this population. Melatonin’s ability to decrease CAMP accumulation caused by dopamine through its D₁ receptors in HEK cells and chick retinal cultures may suggest a possible mechanism for this interaction. In primates, there are two types of horizontal cells, both of which are postsynaptic to cone photoreceptors. The pri mate H₁ horizontal cell subtype is part of the rod phototransduction pathway, in that it is presynaptic to rod and cone photoreceptors, whereas the H₂ subtype is presynaptic only to cones.⁶⁶ That only 18% of horizontal cells in central retina express MT₁ may suggest a limited function for melatonin in this cell type, or alternatively, expression in only one subtype of horizontal cell. If melatonin targets the H₁ horizontal cell, a role for melatonin in rod-dominated vision (i.e., night vision) may be suggested.

One of the unique findings in this study is the presence of MT₁ receptors on photoreceptors. Although melatonin has been implicated in the regulation of rhythmic processes of photoreceptors such as rod cycle shedding and retinomotor movements, receptor expression has not been identified in any mammalian species examined to date and has only just recently been identified in *Xenopus*. The finding of MT₁ expression in photoreceptor cells in the human not only provides the first evidence for the direct action of melatonin on photoreceptors in mammals, but also suggests interspecies variation in melatonin action in the retina. That only rod photoreceptors outside of the most central and most peripheral regions of the human retina express the MT₁ receptor may reflect the heterogeneity of the primate retina. However, further investigation must be conducted before any insight can be gained on the significance of this regional difference in MT₁.
expression. That rods and not cone photoreceptors are melatonin targets may again suggest a role for melatonin in the rod phototransduction pathway. Melatonin is believed to prime the retina at night for rod disc shedding in the early morning, both in lower vertebrates and in rats.\(^{7,8}\) Whether this effect is mediated through the MT\(_1\) receptor remains unknown.

Much of what is known about melatonin function in the retina has been determined in lower vertebrates, species with a fixed pupillary aperture. In these groups, the evidence suggests that melatonin-dopamine interactions may provide a morphologic means of adapting the retina to changes in light intensities.\(^{9,10}\) However, further investigation is necessary to determine how melatonin impacts on mammalian retinal function. The findings in the current study that most dopamine-producing neurons and horizontal, ganglion, and rod photoreceptor cells are all targets for melatonin suggests that melatonin action in the human retina is more complex than previously thought, occurring at multiple levels of phototransduction processing. Further information is required on the subtypes of cells that express MT\(_1\), as well as the distribution of the MT\(_2\) melatonin receptor, to gain a clearer understanding of the function of melatonin in the retina.

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