Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Eye Research 77 (2003) 219–225
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Melatonin receptor expression in the cornea and sclera

Allan F. Wiechmann[a,b,*], Jody A. Rada[a]

[a]*Department of Cell Biology, Health Sciences Center, University of Oklahoma, P.O. Box 26901, Oklahoma City, OK, 73190, USA*
[b]*Department of Ophthalmology, Health Sciences Center, University of Oklahoma, Oklahoma City, OK, 73190, USA*

Received 21 February 2003; accepted in revised form 14 April 2003

Abstract

The scornea and sclera have been shown to exhibit circadian rhythms in cellular proliferation, wound healing and extracellular matrix synthesis. The distribution of melatonin Mel_{1a} and Mel_{1c} receptors was examined in the cornea and sclera of the *Xenopus laevis* eye in order to determine whether melatonin may potentially influence the growth and/or development of these ocular tissues. Sections of adult *X. laevis* eyes were analyzed by immunocytochemistry and confocal microscopy, using antibodies prepared against specific peptide sequences of the *Xenopus* Mel_{1a} and Mel_{1c} receptor proteins. Antibodies were pre- incubated with their appropriate antigenic peptides to control for non-specific labelling. Analysis of the distribution of Mel_{1a} and Mel_{1c} receptor immunoreactivity in the *Xenopus* eye revealed that both the Mel_{1a} and Mel_{1c} receptors were located in the outer fibrous layer (OFL) of the sclera, with Mel_{1c} labelling being the most prominent. Similarly, Mel_{1a} and Mel_{1c} (Mel_{1c} mostly) were also located in cells of the inner fibrous layer (IFL) with Mel_{1c} being most abundant. The chondrocytes of the cartilaginous layer also appeared to express Mel_{1a}, Mel_{1c}, or both receptors. Both Mel_{1a} and Mel_{1c} receptor immunoreactivity were observed in the corneal epithelium and endothelium. Whereas the Mel_{1a} antibody labelled the entire corneal epithelial layer, the Mel_{1c} antibody labelled only the most superficial layer of epithelial cells. Cell processes of fibroblasts of the corneal stroma were immunoreactive for either Mel_{1a} or Mel_{1c} receptors. The identification of Mel_{1a} and Mel_{1c} receptors in restricted distributions in the cornea and sclera suggests that melatonin may play a role in the cellular physiology of these ocular tissues.

Keywords: melatonin; receptor; cornea; sclera; Xenopus laevis; immunocytochemistry; circadian rhythm

1. Introduction

Many physiological processes demonstrate a 24 hr periodicity; these diurnal rhythms may be endogenous (circadian; driven by an internal clock) or they may require the cycle of light and dark to maintain their rhythms. In the eye, diurnal rhythms have been demonstrated at all levels of organization ranging from fundamental molecular events to whole organ/system level processes.

A regular diurnal rhythm of light and dark periods has been shown to be essential for normal corneal growth and development. Exposure to continuous illumination disrupts normal ocular development in young chicks, causing severe corneal flattening, corneal thickening, shallow anterior chambers and progressive hyperopia (Li et al., 1995; Li and Howland, 2000). Local mechanisms appear to play a major role on the constant light- mediated effects on corneal growth, as optic nerve section does not alter the corneal response to constant light (Li et al., 1995). Moreover, the renewal of the corneal epithelium has been shown to exhibit circadian regulation (Doughty, 1990). The mitotic rate of corneal epithelial cells has been shown to be high during the night and low during the day, with circadian variations in epithelial cell proliferation appearing to be most prevalent in peripheral regions of rat corneal epithelium (Oishi and Matsumoto, 1985; Sandvig, Haaskjold, and Refsum, 1994). This circadian variation has been shown to affect the process of corneal epithelial wound healing (Buffa et al., 1993), and influence the action of drugs on the corneal epithelium (Burns and Scheving, 1973; Cardoso and Sowell, 1974).

Strong evidence from animal models indicates that ocular circadian rhythms play a role in the rate of axial

* Corresponding author. Dr Allan F. Wiechmann, Department of Cell Biology, Health Sciences Center, University of Oklahoma, P.O. Box 26901, Oklahoma City, OK 73190, USA.
E-mail address: allan-wiechmann@ouhsc.edu (A.F. Wiechmann).

0014-4835/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
DOI:10.1016/S0014-4835(03)00126-X

elongation and scleral growth. It has been shown in chicks, that axial length fluctuates in a diurnal rhythm, elongating more during the day than during the night (Nickla et al., 1998; Papastergiou et al., 1998; Weiss and Schaeffel, 1993). Under conditions of excessive ocular elongation and myopia, the rate of ocular growth is normal in the daytime; however, the rate of nighttime ocular elongation is higher than normal, resulting in overall increased axial length. Moreover, the sclera of chick eyes shows rhythmic fluctuations in proteoglycan synthesis in vitro with a period of approximately 24 hr (Nickla, et al., 1999), suggesting that rhythmic oscillations in scleral proteoglycan synthesis contribute to the rhythm in axial elongation.

The indoleamine hormone melatonin transmits daily and seasonal timing information to a variety of tissues in essentially all vertebrate species. Melatonin is rhythmically synthesized by pinealocytes, retinal photoreceptors, and ciliary epithelial cells (Cahill and Besharse, 1992; Martin et al., 1992) on a diurnal rhythm, with peak levels occurring in the dark period (Cahill and Besharse, 1992; Cahill et al., 1991). The circadian rhythm of melatonin synthesis and release by retinal cells has been shown to modulate a variety of biological rhythms in the eye, including circadian outer segment disc shedding in photoreceptor cells (Besharse and Iuvone, 1983; White and Fisher, 1989), regulation of horizontal cell sensitivity to light (Wiechmann et al., 1988), modulation of dopamine release (Dubocovich, 1983), photomechanical movements (Pierce and Besharse, 1985) and circadian changes in intraocular pressure (Pintor et al., 2001).

The specific functions of melatonin are mediated by G protein-coupled receptors. Three melatonin receptors (Mel_{1a}, Mel_{1b}, and Mel_{1c}) have been cloned (Ebisawa et al, 1994; Reppert et al., 1995a, b). The Mel_{1a} receptor sequence is homologous to the mammalian MT1 melatonin receptor, and the Mel_{1b} receptor is homologous to the mammalian MT2 receptor. The Mel_{1c} receptor has been cloned from *Xenopus laevis*, chickens, and zebrafish (Reppert et al., 1995b) but an equivalent receptor has not yet been found to be expressed in mammals (Reppert et al., 1995a).

Few studies have examined the role of melatonin in the growth and/or maintenance of non-neuronal tissues, although rhythms in corneal epithelial cell mitotic rate, wound healing and scleral extracellular matrix synthesis have been described (Doughty, 1990; Buffa et al., 1993; Sasaki et al., 1995; Nickla et al., 1999).

Because diurnal rhythms appear to play a role in the regulation of corneal and scleral growth and development, we examined the distribution of Mel_{1a} and Mel_{1c} melatonin receptor immunoreactivity in the cornea and sclera of *X. laevis*. In this report, we demonstrate that melatonin receptors are expressed in cells of the cornea and sclera, and display co-localization and a differential pattern of expression.

2. Materials and methods

2.1. Animals and tissue preparation

Post-metamorphic *X. laevis* (African clawed frogs) were obtained from *Xenopus* Express (Plant City, FL, USA) and maintained in aquaria at 20°C on a daily lighting schedule of 12 hr dark: 12 hr light. Frogs were anesthetized with triciane methylsulfonate (MS-222), and tissues were fixed for 18 hr at 4°C in 4% paraformaldehyde in 0·1 M phosphate buffer pH 7·4, then were transferred to 30% sucrose in phosphate buffer for 16–20 hr at 4°C. Sagittal 30 μm sections were cut on a cryostat microtome and collected on glass slides. The animals were cared for in accordance with the NIH guidelines as described in 'Principles of laboratory animal care', (NIH publication no. 86–23, revised 1985).

2.2. Immunocytochemistry

For immunocytochemical localization of the Mel_{1a} and Mel_{1c} receptors in the cornea and sclera, cryostat sections were rinsed in PBS, and then incubated in incubation buffer (1% normal goat serum (Sigma), 0·2% Triton X-100, and 0·004% sodium azide in PBS) for 30 min at RT. In control experiments, the melatonin receptor antibodies were incubated overnight at 4°C with 1 μM of their corresponding antigen peptides. Sections were then incubated with 2 μg/ml of the Mel_{1c} melatonin receptor antibody in incubation buffer for 3 days at 4°C. Polyclonal antibodies directed against peptides corresponding to regions of the third cytoplasmic loop of the *X. laevis* Mel_{1c} receptor (residues 231–243; KQKLTQTDLRNFL; Wiechmann and Wirsig-Wiechmann, 2001a), and the homologous region of the Mel_{1a} receptor (HHQTWPYNIHGFI; Wiechmann, 2003) were generated in rabbits and chickens, respectively (Research Genetics, Huntsville, AL, USA). Characterization and use of these antibodies have been described previously (Wiechmann and Wirsig-Wiechmann, 2001a; Wiechmann, 2003; Wiechmann et al., 2003).

After the 3-day incubation with the rabbit antibody to the *Xenopus* Mel_{1c} receptor peptide, the sections were rinsed in PBS, and incubated in 5 μg/ml of Alexa Fluor 488 (green) conjugated to goat anti-rabbit antibody (Molecular Probes; Eugene, OR, USA) for 30 min at RT. Sections were rinsed in PBS, then incubated with 2 μg/ml of chicken anti-*Xenopus* Mel_{1a} receptor peptide antibody in PBS for 3 days at 4°C. After rinsing in PBS, sections were incubated in 5 μg/ml of Alexa Fluor 568 (red) conjugated to goat anti-chicken antibody (Molecular Probes) for 30 min at RT. Following another PBS rinse, sections were incubated in 0·0005% DAPI nuclear stain for 10 sec at RT, followed by a final rinse in PBS. Coverslips were mounted onto the slides with Cytoseal 60 mounting matrix (Stephens Scientific, Kalamazoo, MI, USA) and the immunolabeled sections were viewed under an Olympus AX70 fluorescent microscope (Melville, NY, USA) equipped with a Spot colour

digital camera (Diagnostic Instruments, Sterling Heights, MI, USA). For confocal microscopy, sections were viewed under a Zeiss LSM 510 laser-scanning confocal microscope (Jena, Germany). In the specimen shown in this report, the cornea section thickness was 30·1 μm, with 15 sections in the *z*-series, and the sclera section thickness was 14·4 μm, with 18 sections in the *z*-series.

3. Results

We have developed monospecific polyclonal antibodies against the Mel_{1a} and Mel_{1c} melatonin receptors, and have used them in this study to localize Mel_{1a} and Mel_{1c} melatonin receptor expression non-neural ocular tissues in *X. laevis*. The specificity of these antibodies have been described previously, and were used previously to localize these receptors in the frog retina and ciliary epithelium, and the pituitary gland (Wiechmann and Wirsig-Wiechmann, 2001a, b; Wiechmann, 2003; Wiechmann et al., 2003). Melatonin receptor antibodies were raised against peptides corresponding to sequences contained within the third cytoplasmic loop of the *X. laevis* Mel_{1a} and Mel_{1c} receptors (Ebisawa et al., 1994). Using these antibodies, we have detected both Mel_{1a} and Mel_{1c} receptor immunolabeling in the corneal epithelium, stroma and endothelium of *Xenopus* eyes (Fig. 1). The red labelling indicated Mel_{1a} immunoreactivity, and the green label represented Mel_{1c} immunoreactivity. A yellow label indicated areas of Mel_{1a} and Mel_{1c} co- localization, due to the merging of the red and green labels. Mel_{1a} antibodies labelled the entire corneal epithelial layer, whereas Mel_{1c} immunolabeling was limited to the most superficial layer of epithelial cells. Additionally, cell processes of corneal stromal fibroblasts were immunoreactive for either Mel_{1a} or Mel_{1c} receptors.

Mel_{1a} and Mel_{1c} receptor immunoreactivity was also detected in the *Xenopus* sclera, with Mel_{1c} labelling being most intense in the outer fibrous layer (OFL) of the sclera (Fig. 2). Mel_{1a} and Mel_{1c} receptors were also located in cells of the inner fibrous layer (IFL), again with Mel_{1c} exhibiting the strongest labeling. Mel_{1a} and Mel_{1c} immunoreactivity was co- localized in cells of the IFL. Specific labeling for Mel_{1a} and Mel_{1c} in the cornea and sclera was abolished by preincubation of the primary antibodies with the specific antigenic peptides, indicating that immunolabeling for each receptor was specific for each receptor type (Figs. 3(A),(C) and 4(A),(C)). Moreover, preincubation of the Mel_{1a} antibody with the Mel_{1c} antigenic peptide did not diminish the Mel_{1a} immunoreactivity, and visa versa, indicating that the Mel_{1a} and Mel_{1c} antibodies do not cross-react with each other (Figs. 3(B),(D) and 4(B),(D)).

Laser scanning confocal analysis of sections of *Xenopus* cornea and sclera with the Mel_{1a} and Mel_{1c} antibodies revealed more precise localization of receptor localization. Both differential distribution and co-localization was observed in many cells of these tissues. In the cornea, Mel_{1a} and Mel_{1c} immunoreactivity was co- localized to the superficial layers of epithelial cells in the corneal epithelium, whereas only Mel_{1a} immunoreactivity was also located in the deeper layers of epithelial cells in the corneal epithelium (Fig. 5). Mel_{1a} and Mel_{1c} co-localization was observed in the endothelial cells (Fig. 5). In the corneal stroma, Mel_{1a} immunoreactivity was present in the fibroblasts, and a very small number of fibroblasts appeared to also express some Mel_{1c} immunoreactivity. Mel_{1c} immunoreactivity alone (i.e.; without Mel_{1a}) was not observed in the fibroblasts of the corneal stroma. Furthermore,

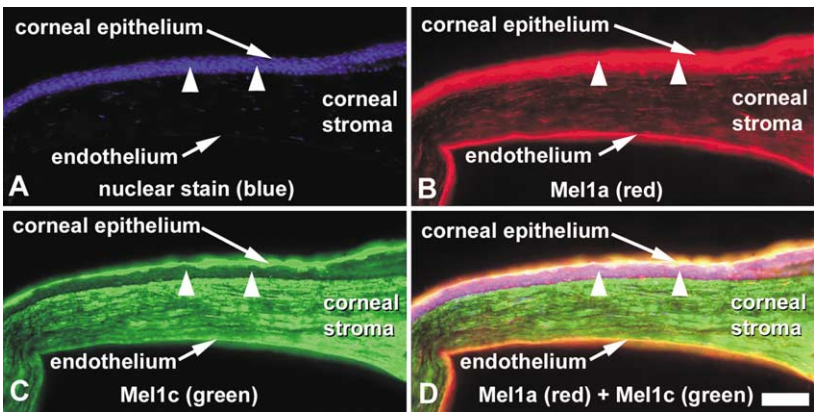

Fig. 1. Immunocytochemistry of *Xenopus laevis* cornea with melatonin Mel_{1a} and Mel_{1c} receptor antibodies. (A) Corneal section stained with blue nuclear dye (DAPI). (B) Corneal section incubated with Mel_{1a} receptor antibody followed by incubation in secondary antibody conjugated to a red fluorescent dye. Mel_{1a} labelling is intense in corneal epithelium (arrowheads) and endothelium, and to a lesser extent labels cells in the corneal stroma. (C) Corneal section incubated with Mel_{1c} receptor antibody followed by incubation in secondary antibody conjugated to a green fluorescent dye. Mel_{1c} labelling is intense in the superficial layer of corneal epithelium and in cells of the corneal stroma. The corneal endothelium and deeper layers of epithelium (arrowheads) were weakly stained. (D) Merged image of Mel_{1a} and Mel_{1c} labelled cornea. Yellow color indicates areas of co- localization of Mel_{1a} and Mel_{1c}. Scale bar = 100 μm.

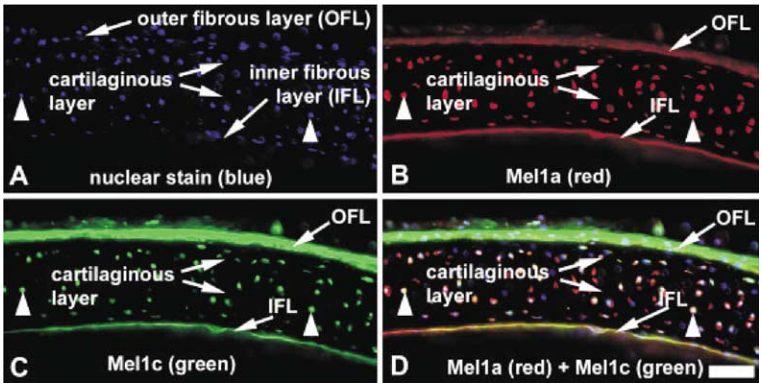

Fig. 2. Immunocytochemistry of *Xenopus laevis* sclera with melatonin Mel_{1a} and Mel_{1c} receptor antibodies. (A) Scleral section stained with blue nuclear dye (DAPI). (B) Scleral section incubated with Mel_{1a} receptor antibody followed by incubation in secondary antibody conjugated to a red fluorescent dye. Mel_{1a} labelling is present in the outer fibrous layer (OFL), the inner fibrous layer (IFL), and on chondrocytes in the cartilaginous layer (arrowheads). (C) Scleral section incubated with Mel_{1c} receptor antibody followed by incubation in secondary antibody conjugated to green fluorescent dye. Mel_{1c} labelling is most intense in the outer fibrous layer (OFL) and is also present on the inner fibrous layer (IFL) and on chondrocytes in the cartilaginous layer (arrowheads). D) Merged image of Mel_{1a} and Mel_{1c} labelled sclera. Yellow colour indicates areas of co- localization of Mel_{1a} and Mel_{1c}. Scale bar = 100 μm.

the collagen matrix was not labelled substantially with either antibody and the laser scanning analysis revealed that the apparent matrix labelling by the Mel_{1c} antibody seen in Figs. 1 and 2 was the result of artifactual non-specific labelling.

In the sclera, Mel_{1a} and Mel_{1c} immunoreactivity was co-localized to the deeper layers of the OFL, and some Mel_{1a} immunoreactivity appeared to be located also in the superficial layers of the OFL (Fig. 5). In the IFL, Mel_{1a} and Mel_{1c} immunoreactivity was co-localized (Fig. 5). In the hyaline cartilage of the cartilaginous layer, Mel_{1a} and Mel_{1c} immunoreactivity displayed both co-localization and differential expression in the chondrocytes. The cartilage matrix was not labeled with either antibody.

4. Discussion

The results of this study provide new data on the localization of melatonin receptors, Mel_{1a} and Mel_{1c} in the corneal epithelium, stroma and endothelium of *Xenopus eyes*, as well as in the outer and IFL of the sclera. The identification of melatonin receptors on the cornea and sclera of the *Xenopus* eye suggests that cells in these tissues may be target sites for melatonin action, and that some cellular activities in these tissues may be influenced by the circadian exposure to melatonin.

This study has shown that all the major cell types of the cornea express Mel_{1a} and/or Mel_{1c} melatonin receptors. The parallels between the retinal melatonin rhythm and

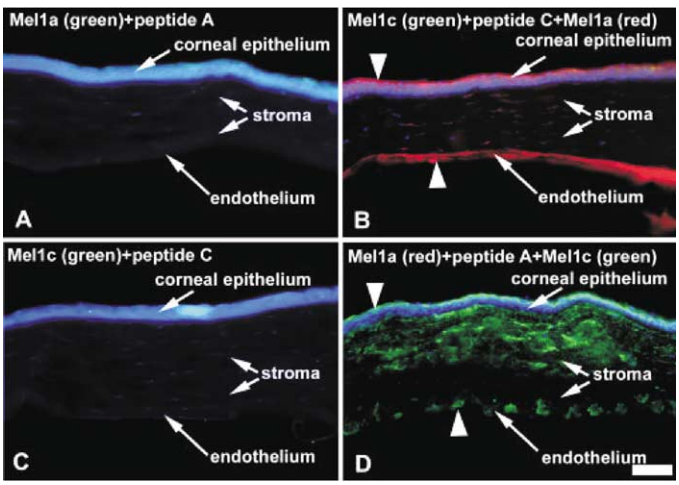

Fig. 3. Peptide block controls for Mel_{1a} and Mel_{1c} labelling in the *Xenopus laevis* cornea. (A and C) Corneal sections were incubated with green fluorescent dye conjugated Mel_{1a} or Mel_{1c} receptor antibodies previously pre-incubated with their corresponding peptide and were counterstained with DAPI blue nuclear dye. Absence of green stain indicates complete block of specific antibody binding with specific peptide. (B and D) Corneal sections were incubated with red fluorescent dye-conjugated Mel_{1a} receptor antibody together with green fluorescent dye n conjugated Mel_{1c} receptor antibody previously pre- incubated with either Mel_{1a} or Mel_{1c} peptide and counterstained with DAPI blue nuclear dye. Red labelling indicates areas of specific Mel_{1a} receptor labelling in epithelium and green labelling indicates areas of specific Mel_{1c} receptor labelling in surface epithelium, endothelium (arrowheads) and stroma. Scale bar = 100 μm.

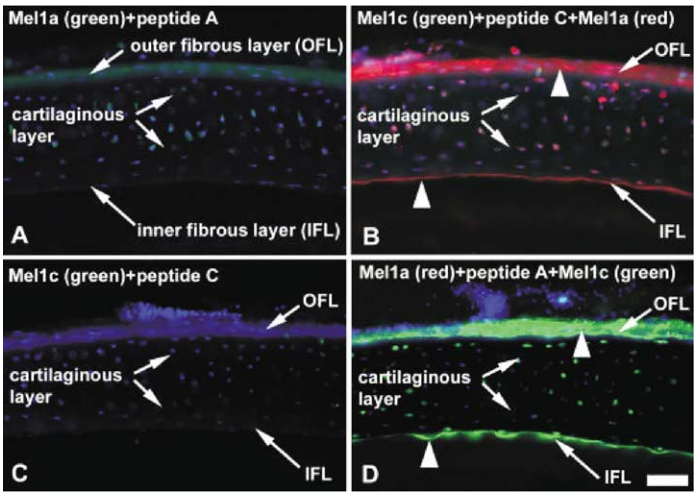

Fig. 4. Peptide block controls for Mel_{1a} and Mel_{1c} labelling in the *Xenopus laevis* sclera. (A and C) Scleral sections were incubated with green fluorescent dye conjugated Mel_{1a} or Mel_{1c} receptor antibodies previously pre- incubated with their corresponding peptide and were counterstained with DAPI blue nuclear dye. Absence of green stain indicates complete block of specific antibody binding with specific peptide. (B and D) Scleral sections were incubated with red fluorescent dye-conjugated Mel_{1a} receptor antibody together with green fluorescent dye conjugated Mel_{1c} receptor antibody previously pre-incubated with either Mel_{1a} or Mel_{1c} peptide and counterstained with DAPI blue nuclear dye. Red labelling indicates areas of specific Mel_{1a} receptor labelling in outer and inner fibrous layers (OFL and IFL; arrowheads) as well as in the cartilaginous layer and green labelling indicates areas of specific Mel_{1c} receptor labelling in outer and inner fibrous layers (OFL and IFL; arrowheads) and in the cartilaginous layer. Scale bar = 100 μm.

the rhythms in corneal epithelial mitotic activity (Sasaki et al., 1995) suggest a role for melatonin in corneal growth and repair. The renewal of the corneal epithelium has been shown to exhibit a circadian regulation in the mitotic rate of corneal epithelial cells, which is high during the night and low during the day (Doughty, 1990). This circadian variation affects the rate of corneal epithelial wound healing (Buffa et al., 1993), and influences the action of drugs on the corneal epithelium (Burns and Scheving, 1973; Cardoso and Sowell, 1974). Moreover, melatonin injection induces a phase advance of the corneal mitotic rhythm in normal light/dark conditions (Sasaki et al., 1995). It is unlikely that melatonin synthesis by retinal photoreceptors is responsible for mediating the corneal mitotic rhythm, since the rhythm is maintained following photoreceptor destruction (Oishi et al., 1996). However, melatonin synthesis by ciliary epithelial cells (Martin et al., 1992) may be responsible for mediating circadian rhythms in corneal growth, maintenance and repair via interactions with specific melatonin receptor subtypes on corneal epithelial, stromal and/or endothelial cells, and corneal epithelial mitotic rates. It has been reported recently that melatonin receptors are expressed in corneal endothelial cells and the basal corneal epithelial cells in the human cornea (Meyer et al., 2002) which is consistent with our findings of Mel_{1a} and Mel_{1c} receptor expression in the corneal endothelium of *X. laevis*.

Our findings that Mel_{1a} and Mel_{1c} receptors are expressed in the fibrous layers of the sclera and to a lesser extent in the cartilaginous layer, suggest that melatonin plays a role in the differential regulation of cellular functions in these scleral layers. In the chick, the fibrous and cartilaginous scleral layers have been shown to undergo opposite responses in extracellular matrix synthesis and degradation under conditions of accelerated and decelerated ocular growth (Rada, et al., 1999; Marzani and Wallman, 1997). We speculate that melatonin, through its actions on distinct melatonin receptor subtypes may play a role in the differential regulation of the growth and remodelling of the fibrous and cartilaginous scleral layers, which in turn would dramatically affect eye size and refraction.

Diurnal melatonin rhythms and melatonin content in the retina have been reported to be unchanged during the development of deprivation myopia despite the breakdown of both diurnal growth rhythms of the eye and the diurnal rhythm in retinal dopamine metabolism (Hoffman and Schaeffel, 1996). However, intravitreal injection of melatonin slightly suppresses deprivation myopia, whereas depletion of retinal serotonin, an intermediate product in the synthesis of melatonin, enhances deprivation myopia (Hoffman and Schaeffel, 1996). The identification of melatonin receptors on fibroblasts and chondrocytes of the *Xenopus* sclera in the present study, together with results from previous studies indicating that diurnal rhythms play a role in regulating the rate of ocular elongation (Nickla et al., 1998; Papastergiou et al., 1998; Weiss and Schaeffel, 1993), provides compelling evidence for the role of melatonin and/or melatonin receptor expression in the regulation of ocular growth and refraction.

Very little is known about the functional roles of the melatonin receptors in controlling ocular growth and repair. Intravitreal application of the competitive melatonin receptor luzindole antagonist has been shown to inhibit

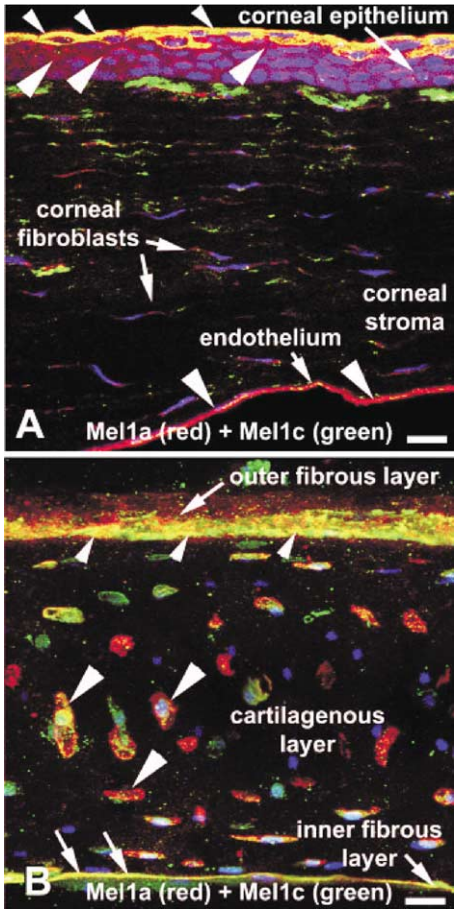

Fig. 5. Confocal image of the differential distribution and co- localization of Mel_{1a} and Mel_{1c} receptor immunoreactivity in the *Xenopus laevis* cornea and sclera. Sections of *Xenopus* eyes were incubated first with the Mel_{1c} receptor antibody and labelled with a green fluorescent dye-antibody conjugate then incubated with the Mel_{1a} antibody labelled with a red fluorescent dye-antibody conjugate and analyzed by confocal microscopy. The merged image of Mel_{1c} and Mel_{1a} immunolabeling demonstrates the differential expression and co-localization of the two receptor types. (A) The two receptors appear to have some co- localization in the superficial layers of the corneal epithelium (small arrowheads), as indicated by the yellow fluorescence. Only the Mel_{1a} red immunolabeling appears in the deeper layers of the corneal epithelium (large arrowheads). Some specific Mel_{1c} green immunolabeling appears in corneal fibroblasts (small arrows) and in the corneal endothelium (large arrowheads), but the labelling that occurs in the non-cellular corneal stroma was determined to be artifact. In additional its expression in the corneal epithelium, Mel_{1a} is present in the corneal stroma fibroblasts (small arrows) and the corneal endothelium (large arrowheads). (B) The two receptors are co- localized in the deep layers of the outer fibrous layer of the sclera (small arrowheads), as indicated by the yellow fluorescence. Mel_{1a} and Mel_{1c} are also co- localized to the inner fibrous layer of the sclera (small arrows). The matrix of the cartilaginous layer is not immunoreactive with the antibodies, whereas the chondrocytes (large arrows) display co- localization and/or differential expression of Mel_{1a} and Mel_{1c}. Scale bar = 20 μm.

the melatonin-mediated reduction in dopamine release from retinal neurons (Iuvone et al., 1991; Dubocovich, 1988), protect the eye from light damage to retinal photoreceptors (Sugawara et al., 1998) as well as to block the reduction of intraocular pressure mediated by melatonin agonists (Pintor et al., 1998). Based on the results of the present study, which localize melatonin receptors to principal cells of the cornea and sclera, we hypothesize that melatonin, through int eractions with specific melatonin receptors located on a variety of ocular tissues, modulates circadian rhythms in corneal repair and ocular elongation. Alterations in melatonin and/or melatonin receptors in neural and non-neural eye tissues may lead to impaired wound healing, disorders of ocular growth, and refractive anomalies. The results reported here may provide a stimulus for further studies into previously unknown roles for melatonin and melatonin receptors in non-neural ocular tissues and may bring us closer to understanding the molecular mechanisms underlying ocular growth regulation and repair.

Acknowledgements

This work was supported by OCAST award HR98-049 from the Oklahoma Center for Science and Technology (AFW). The authors thank Melissa Vrieze, Rajendra Dhote, Radhika Dighe, and Courtney McClerran for excellent technical assistance. Ben Fowler of the Oklahoma Medical Research Foundation assisted with the confocal microscopy. This research was supported by NIH grants EY13686 (AFW), EY12191 (NEI Core), RR017713 (COBRE), EY 093919 (JAR), the Oklahoma Center for Science and Technology (OCAST: HR02-135R), and the Presbyterian Health Foundation.

References

Besharse, J.C., Iuvone, P.M., 1983. Regulation of indoleamine N-acetyltransferase activity in the retina: effects of light and dark, protein synthesis inhibitors and cyclic nucleotide analogs. Brain Res. 273, 111–119.

Buffa, A., Rizzi, E., Falconi, M., Matteucci, A., Baratta, B., Fantazzini, A., Lattanzi, G., Rizzoli, R., 1993. Bromodeoxyuridine incorporation in corneal epithelium: an immunocytochemical study in rats. Boll. Soc. Ital. Biol. Sper. 69, 767–773.

Burns, E.R., Scheving, L.E., 1973. Isoproterenol- induced phase shifts in circadian rhythm of mitosis in murine corneal epithelium. J. Cell. Biol. 56, 605–608.

Cahill, G.M., Besharse, J.C., 1992. Light-sensitive melatonin synthesis by *Xenopus* photoreceptors after destruction of the inner retina. Vis. Neurosci. 8, 487–490.

Cahill, G.M., Grace, M.S., Besharse, J.C., 1991. Rhythmic regulation of retinal melatonin: Metabolic pathways, neurochemical mechanisms, and the ocular circadian clock. Cell. Mol. Neurobiol. 11, 529–560.

Cardoso, S.S., Sowell, J.G., 1974. Control of cell division in the cornea of rats. Interaction between isoproterenol and dexamethasone. Proc. Soc. Exp. Biol. Med. 147, 309–313.

Doughty, M.J., 1990. Morphometric analysis of the surface cells of rabbit corneal epithelium by scanning electron microscopy. Am. J. Anat. 189, 316–328.

Dubocovich, M.L., 1983. Melatonin is a potent modulator of dopamine release in the retina. Nature 306, 782–784.

Dubocovich, M.L., 1988. Luzindole (N-0774): a novel melatonin receptor antagonist. J. Pharmacol. Exp. Ther. 246, 902–910.

Ebisawa, T., Karne, S., Lerner, M.R., Reppert, S.M., 1994. Expression cloning of a high-affinity melatonin receptor from Xenopus dermal melanophores. Proc. Nat. Acad. Sci. USA 91, 6133–6137.

Hoffman, M., Schaeffel, F., 1996. Melatonin and deprivation myopia in chickens. Neurochem. Int. 28, 95–107.

Iuvone, P.M., Tigges, M., Stone, R.A., Lambert, S., Laties, A.M., 1991. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. Invest. Ophthalmol. Vis. Sci. 32, 1674–1677.

Li, T., Howland, H.C., 2000. Modulation of constant light effects on the eye by ciliary ganglionectomy and optic nerve section. Vision Res. 40, 2249–2256.

Li, T., Troilo, D., Glasser, A., Howland, H.C., 1995. Constant light produces severe corneal flattening and hyperopia in chickens. Vision Res. 35, 1203–1209.

Martin, X.D., Malina, H.Z., Brennan, M.C., Hendrickson, P.H., Lichter, P.R., 1992. The ciliary body—The third organ found to synthesize indolamines in humans. Eur. J. Ophthalmol. 2, 67–72.

Marzani, D., Wallman, J., 1997. Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. Invest. Ophthalmol. Vis. Sci. 38, 12726–12739.

Meyer, P., Pache, M., Loeffler, K.U., Brydon, L., Jockers, R., Flammer, J., Wirz-Justice, Savaskan, E., 2002. Melatonin MT-1- receptor immunoreactivity in the human eye. Br. J. Ophthalmol. 86, 1053–1057.

Nickla, D.L., Rada, J.A., Wallman, J., 1999. Isolated chick sclera shows a circadian rhythm in proteoglycan synthesis perhaps associated with the rhythm in ocular elongation. J. Comp. Physiol. A 185, 81–90.

Nickla, D.L., Wildoset, C., Wallman, J., 1998. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. Exp. Eye Res. 66, 163–181.

Oishi, T., Matsumoto, M., 1985. Circadian mitotic rhythm in the corneal epithelium of Japanese quail: Intraocular initiation of the rhythm. In: Hiroshige, T., Honma, K. (Eds.), Circadian Clock and Zeitgebers, Hokkaido University, Sapporo, pp. 45–54.

Papastergiou, G.I., Schmid, G.F., Riva, C.E., Mendel, M.J., Stone, R.A., Laties, A.M., 1998. Ocular axial length and choroidal thickness in newly hatched chicks and one-year-old chickens fluctuate in a diurnal pattern that is influenced by visual experience and intraocular pressure changes. Exp. Eye Res. 66, 195–205.

Pierce, M.E., Besharse, J.C., 1985. Circadian regulation of retinomotor movements. I. Interaction of melatonin and dopamine in the control of cone length. J. Gen. Physiol. 86, 6716–6789.

Pintor, J., Martin, L., Pelaez, T., Hoyle, C.H., Peral, A., 2001. Involvement of melatonin MT(3) receptors in the regulation of intraocular pressure in rabbits. Eur. J. Pharmacol. 416, 251–254.

Rada, J.A., Perry, C.A., Slover, M.L., Achen, V.R., 1999. Gelatinase A and TIMP-2 expression in the fibrous sclera of myopic, recovering and control chick eyes. Invest. Ophthalmol. Vis. Sci. 40, 3091–3099.

Reppert, S.M., Godson, C., Mahle, C.D., Weaver, D.R., Slaugenhaupt, S.A., Gusella, J.F., 1995a. Molecular characterization of a second melatonin receptor expressed in human retina and brain: The Mel_{1b} melatonin receptor. Proc. Nat. Acad. Sci. USA 92, 8734–8738.

Reppert, S.M., Weaver, D.R., Cassone, V.M., Godson, C., Kolakowski, L.F., 1995b. Melatonin receptors are for the birds: Molecular analysis of two receptor subtypes differentially expressed in chick brain. Neuron 15, 1003–1015.

Sandvig, K.U., Haaskjold, E., Refsum, S.B., 1994. Time dependency in the regenerative response to injury of the rat corneal epithelium. Chronobiol. Int. 11, 173–179.

Sasaki, M., Masuda, A., Oishi, T., 1995. Circadian rhythms of corneal mitotic rate, retinal melatonin and immunoreactive visual pigments, and the effects of melatonin on the rhythms in the Japanese quail. J. Comp. Physiol. A 176, 465–471.

Sugawara, T., Sieving, P.A., Iuvone, P.M., Bush, R.A., 1998. The melatonin antagonist luzindole protects retinal photoreceptors from light damage in the rat. Invest. Ophthalmol. Vis. Sci. 39, 2458–2465.

Weiss, S., Schaeffel, F., 1993. Diurnal growth rhythms in the chicken eye: relation to myopia development and retinal dopamine levels. J Comp Physiol A 172, 263–270.

White, M.P., Fisher, L.J., 1989. Effects of exogenous melatonin on circadian disc shedding in the albino rat retina. Vision Res. 29, 167–179.

Wiechmann, A.F., 2003. Differential distribution of Mel_{1a} and Mel_{1c} melatonin receptors in *Xenopus laevis* retina. Exp. Eye Res. 76, 1–8.

Wiechmann, A.F., Vrieze, M.J., Wirsig-Wiechmann, C.R., 2003. Differential distribution of melatonin receptors in the pituitary gland of *Xenopus laevis*. Anat. Embryol. 206, 291–299.

Wiechmann, A.F., Wirsig-Wiechmann, C.R., 2001a. Multiple cell targets for melatonin action in *Xenopus laevis* retina: distribution of melatonin receptor immunoreactivity. Vis. Neurosci. 18, 695–702.

Wiechmann, A.F., Wirsig-Wiechmann, C.R., 2001b. Melatonin receptor mRNA and protein expression in *Xenopus laevis* nonpigmented ciliary epithelial cells. Exp. Eye Res. 73, 617–623.

Wiechmann, A.F., Yang, X.L., Wu, S.M., Hollyfield, J.G., 1988. Melatonin enhances horizontal cell sensitivity in salamander retina. Brain Res. 453, 377–380.