Rosenstein, Ruth E.; Srinivasan, Venkataramanujan; Spence, D. Warren,; Pandi-Perumal, Seithikurippu R.; Brown, Gregory M.; Cardinali, Daniel P.

Melatonin as a therapeutic tool in ophthalmology : implication for glaucoma and uveitis

Preprint del documento publicado en Journal of Pineal Research 49(1) 1-13; 2010

(Publicado en Journal of Pineal Research 2010 49(1):1-13)

Melatonin as a therapeutic tool in ophthalmology: implication for glaucoma and uveitis

Ruth E. Rosenstein.[1,CA] Seithikurippu R. Pandi-Perumal,[2] Venkataramanujan Srinivasan,[3] D. Warren Spence,[4] Gregory M. Brown[5], Daniel P. Cardinali[6]

[1]Department of Human Biochemistry, School of Medicine, CEFyBO, University of Buenos Aires, CONICET, Buenos Aires, Argentina, [2]Somnogen Inc, 8790 112th Street, New York, NY 11418-2317, USA, [3]Sri Sathya Sai Medical Education and Research Foundation, Prasahsnthi Nilayam, 40 Kovai Thirunagar, Civil Aerorome Post Coimbatore 641014, India, [4]Sleep and Alertness Clinic, Toronto, Ontario, M1S 1T7, Canada, [5]Department of Psychiatry, School of Medicine, University of Toronto, 100 Bronte Road. Oakville, ON L6L 6L5, Canada, [6]Departament of Teaching & Research, School of Medical Sciences, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina.

Running title:

Melatonin in glaucoma and uveitis

Corresponding Author:

Dr. Ruth E. Rosenstein
Department of Human Biochemistry,
School of Medicine, CEFyBO
University of Buenos Aires, CONICET
Paraguay 2155, 5th floor, (1121)
Buenos Aires, ARGENTINA
Tel: 54-11-45083672 (ext 37)
Fax: 54-11-45083672 (ext 317)
e-mail: ruthr@fmed.uba.ar

Abbreviations used

5-MCA-NAT, 5- methoxycarbonylamino-N- acetyltryptamine; AANAT, arylalamine N acetyl transferase; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; AMK, N^1-acetyl-5-methoxykynuramine; ERG, electroretinogram; HA, hyaluronic acid; HIOMT, hydroxyindole-*O*-

methyltransferase; IOP, intraocular pressure; RGCs, retinal ganglion cells; RPE, retinal pigment epithelium; SCN, suprachiasmatic nuclei; BOB, blood-ocular barrier; BDNF, brain-derived neurotrophic factor; Abbreviations for the melatonin receptors are from the IUPHAR database (Harmar et al., 2009)

Table of contents

Abstract

Several lines of evidence support the view that increased free radical generation and altered nitric oxide (NO) metabolism play a role in the pathogenesis of highly prevalent ocular diseases, such as glaucoma and uveitis. Data are discussed indicating that melatonin, being an efficient antioxidant that displays antinitridergic properties, has a promising role in the treatment of these ocular dysfunctions. Melatonin synthesis occurs in the eye of most species and melatonin receptors are localized in different ocular structures. In view of the fact that melatonin lacks significant adverse collateral effects even at high doses, the application of melatonin could potentially protect ocular tissues by effectively scavenging free radicals and excessive amounts of NO generated in the glaucomatous or uveitic eye.

1. Background

Melatonin is a ubiquitous natural substance which is widely distributed in nature, being found both in plants and animals (Pandi-Perumal et al., 2006; Reiter et al., 2007). Melatonin is probably one of the first biologically significant compounds that appeared in living organisms. Although in all mammals including humans, melatonin is primarily synthesized in the pineal gland, its synthesis also occurs in other tissues, such as bone marrow, gut, gastrointestinal tract, lymphocytes, and in various parts of the eye in most mammals, i.e. the retina (Cardinali & Rosner, 1971a, 1971b; Tosini & Menaker, 1998), the ciliary body (Martin et al., 1992) and the lachrymal gland (Mhatre et al., 1988).

In the eye, melatonin which is locally synthesized or entering from the circulation may contribute to the regulation of retinomotor movements, rod outer segment disc shedding, dopamine synthesis and release, and intraocular pressure (IOP). Moreover, melatonin is an effective antioxidant and free radical scavenger (Siu et al., 2006; Lundmark et al., 2006; Lundmark et al., 2007; Alarma-Estrany & Pintor, 2007) which protects the photoreceptor outer segment from oxidative damage induced by light (Siu et al., 1999; Marchiafava & Longoni, 1999).

Glaucoma is a complex disease with a number of risk factors and mechanisms which lead to retinal ganglion cell (RGCs) death. Although an increase in IOP definitely plays a causal role in glaucomatous neuropathy, other factors such as a reduced antioxidant defense system activity and an increase in the nitridergic pathway activity, have been suggested as possible additional causes for early stage of glaucomatous damage (Bunin et al., 1992; Neufeld , 1999; Moreno et al., 2004; Belforte et al., 2007). The current management of glaucoma is mainly directed at the control of IOP; however, a therapy that prevents the death of ganglion cells should be the main goal of treatment.

Uveitis is a common ophthalmic disorder characterized by an acute, recurrent, or persistent ocular inflammation. Uveitis is associated with the disruption of the blood-ocular barrier (BOB) as well as protein leakage and leukocyte infiltration into the aqueous humor. Several lines of evidence support the view that uveitis results from damage generated by infiltrated leukocytes which release cytokines and other inflammatory chemical mediators (de Vos et al., 1994), including arachidonic acid (AA) metabolites (Bellot et al., 1996), reactive oxygen species (ROS) (Sasaki et al., 2009), NO (Jacquemin et al., 1996), and superoxide anion (Hashida et al., 2000), among many others. At present, the therapy for uveitis generally based on the use of corticoids, is essentially directed towards pain reduction and to avoid lesions of ocular tissues (de Vos et al., 1994; Nathan & Xie, 1994; Jacquemin et al., 1996; Hashida et al., 2000; Sasaki et al., 2009). However, the immunosuppressive effect of corticoids may contribute to the development of systemic disease. Moreover, this strategy carries with it the attendant risk of cortisonic glaucoma induction that usually follows its chronic use (Nathan & Xie, 1994).

Despite the fact that uveitis and glaucoma are highly prevalent and major causes of blindness, there are currently no optimal treatments for these ocular diseases. In recent years, melatonin has been identified as a neuroprotector in experimental animal models of various neurological and neurodegenerative disorders (Reiter et al., 1999; Srinivasan et al., 2005, 2006). In this context, we will consider evidence supporting that melatonin should be regarded as an important ophthalmic therapeutic resource, particularly for the management of glaucoma and uveitis.

2. Melatonin biosynthesis in the eye

In most mammals, melatonin is synthesized intraocularly through the same pathway which occurs in the pineal gland (Axelrod, 1974). Tryptophan taken up from the blood is converted into serotonin, which is subsequently metabolized to N-acetyl serotonin by the enzyme arylalkylamine N-acetyltransferase (AA-NAT). N-acetyl serotonin is then converted into melatonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT). However, since HIOMT is virtually lacking in the human retina, the major end product of this synthetic pathway may be N-acetylserotonin rather than melatonin (Rodriguez et al., 1994; Bernard et al., 1995).

The earliest finding supporting melatonin's biosynthetic pathway in the mammalian retina was the description of HIOMT activity (Cardinali & Rosner, 1971a) and the demonstration that labeled serotonin is converted into melatonin in the rat retina (Cardinali & Rosner, 1971b). The presence of HIOMT in the chicken retina at both protein and mRNA level has been confirmed (Bernard et al., 1999; Liu et al., 2004). The gene encoding HIOMT is selectively expressed in retinal photoreceptors. In a recent study, the promoter of HIOMT gene Otx2 was detected both in the chicken retina and pineal gland (Dinet et al., 2006). These findings are significant in view of the fact that Otx2 protein is present at "the right place and at the right time" to play a role in the onset of HIOMT gene expression in retinal photoreceptors and pineal gland (Dinet et al., 2006). AA-NAT levels show a circadian rhythm, peaking at night in the chicken and rat retina (Niki et al., 1998; Iuvone et al., 2002). The presence of AA-NAT in the human eye has been well documented (Coon et al., 1996). In the rhesus monkey, AA-NAT activity in pineal and retina shows more than a four-fold increase at night (Coon et al., 2002).

Melatonin biosynthesis in the retina of the golden hamster is regulated by the light/dark cycle (Faillace et al., 1995). In addition, the finding that isolated *Xenopus* photoreceptor cells rhythmically secrete melatonin suggests that photoreceptors contain an endogenous circadian clock which regulates melatonin biosynthesis (Cahill & Besharse, 1993). This hypothesis has been confirmed in the golden hamster and mouse retinas (Tosini & Menaker, 1996, 1998). In fact, several genes identified as components of the core oscillator in the suprachiasmatic nuclei (SCN) have also been localized in the retina. The clock genes cryptochrome 1 and 2 are expressed in chicken inner retinal neurons and in the ganglion cell layer (Haque et al., 2002; Bailey et al., 2002). Furthermore, Period 1 and Period 2 have been identified in the rat retina inner nuclear layer (Namihira et al., 2001), and in a few ganglion cells of the mouse and human retina (Witkovsky et al., 2003; Thompson et al., 2004). At present, it is not known whether the circadian rhythms in mammalian photoreceptors are driven by inner retinal clocks through synaptic ribbons or by paracrine outputs. Experiments with the rodless mouse have shown that melatonin synthesis is not abolished by the complete loss of photoreceptors but that its circadian expression disappears (Tosini & Menaker, 1998; Tosini, 2000). This finding suggests that rods are necessary for the rhythmic synthesis of melatonin. However, new evidences indicate that chick retinal ganglion cells are able to rhythmically synthesize melatonin, even in isolated conditions (Garbarino-Pico et al., 2004).

Regulated by the interaction between a circadian clock and the photic information, retinal melatonin levels rise rapidly after the onset of darkness and decrease after light exposure in the golden hamster and rat (Faillace et al., 1995; Fukuhara et al., 2001). The entire sequence of events has been elegantly studied in chicken photoreceptor cells. The depolarization of photoreceptors that occurs during darkness induces AA-NAT activity by a Ca^{2+} and cAMP dependent mechanism (Ivanova & Iuvone, 2003). Depolarization of the photoreceptor membrane opens dihydropyridine sensitive voltage-gated Ca^{2+} channels resulting in sustained increases in intracellular Ca^{2+} concentrations in the inner segments of photoreceptors, which in turn stimulate cAMP formation through activation of calmodulin dependent adenyl cyclase (Gan et al., 1995). Increased levels of cAMP induce AA-NAT gene transcription and increase its activity causing an augmented production of melatonin (Alonso-Gomez & Iuvone, 1995; Greve et al., 1999).

Stability of AA-NAT is regulated by cAMP, and light, by decreasing cAMP levels in photoreceptor cells, results in rapid degradation of AA-NAT protein by proteasomal proteolysis (Tosini et al., 2006).

As for the regulation of melatonin biosynthesis by a circadian clock, the gating process involves an E box-mediated transcriptional activation of the adenyl cyclase gene. This regulates melatonin synthesis by regulating the expression of type 1 adenyl cyclase, and the synthesis of cAMP in photoreceptors (Fukuhara et al., 2004). Cyclic AMP signaling may play a key role in the input and output components of the central circadian axis, the retina, the SCN, and the pineal gland (Fukuhara et al., 2004). In addition to cAMP, binding of AA-NAT to 14-3-3 proteins in retinal photoreceptors appears to be important in the response to light and darkness (Pozdeyev et al., 2006). During darkness, retinal AA-NAT is phosphorylated and forms a complex with 14-3-3 proteins that protects it from dephosphorylation and degradation. Binding of AA-NAT to 14-3-3 proteins is disrupted by light in photoreceptors which allows AA-NAT degradation. Although the studies cited above have been conducted in the chicken, several *in vivo* and *in vitro* studies have established that there is a circadian clock system in the mammalian eye independent from the SCN (Tosini et al., 2008).

Besides the retina, it has been reported that both AA-NAT and HIOMT are present in other ocular structures such as rabbit and rat lens (Abe et al., 1999; Itoh et al., 2007).

Despite the foregoing discussion on melatonin synthesis in the eye, it is doubtful that significant melatonin synthesis occurs in the retina of either the human or the rhesus monkey. The enzyme HIOMT is present in insignificant quantities in those species. Not only is HIOMT protein and activity undetectable but HIOMT mRNA is detectable only by the most highly sensitive methodology (Rodriguez et al., 1994; Bernard et al., 1995; Coon et al., 2002). It should be noted, however, that the amount of melatonin synthesis required for a secreting gland is of a much greater magnitude than for a tissue where melatonin acts locally. Thus, if a small amount of synthesis were to occur, that might be sufficient to promote activity at the local level. In addition, all the preceding steps in the synthetic pathway are present in these tissues, so that synthesis of N-acetylserotonin undoubtedly shows a huge increase shortly after dark onset and drops precipitously at light onset in humans as well as rhesus monkeys. Thus, N-acetylserotonin might conceivably substitute for melatonin or have another role such acting as an antioxidant (see below) in the primate eye. Finally, in the human and in the rhesus monkey, circulating melatonin is capable of diffusing into the eye and could potentially act on melatonin receptors. That is possible because melatonin is highly lipophilic and readily diffuses into tissues. In that event, in humans and rhesus monkeys, unlike other mammals such as the rat, the rhythm of activation of melatonin receptors in the eye could be totally dependent on the pineal gland.

3. Melatonin receptors in the eye

Since most of melatonin functions are attributed to its interaction with specific receptors, the study of the distribution of melatonin receptor subtypes in the eye assumes functional significance (Alarma-Estrany & Pintor, 2007). Immunocytochemical analysis of ocular tissues obtained from various species, including chickens, rats, and humans, shows that melatonin receptors MT1 and MT2 (formerly Mel_{1a} and Mel_{1b}, respectively) are localized in the cornea, choroid, sclera, retina, and retinal blood vessels (Ascher et al., 1995; Fujieda & Hamadanizadeh, 1999; Scher et al., 2002; Savaskan et al., 2002b; Scher et al., 2003; Wiechmann et al., 2004; Rada & Wiechmann, 2006), as shown in Figure 1. In *Xenopus* eyes, MT1 melatonin receptors have been described in the corneal epithelium, stroma, sclera, and endothelium (Wiechmann & Rada, 2003), suggesting melatonin's involvement in the differential regulation of growth and remodeling of the fibrous and cartilaginous scleral layers that affect eye size and refraction. On

the other hand, the localization of melatonin receptors in the iris and ciliary processes could indicate that they may be involved in regulating IOP (Osborne et al., 1999). The presence of melatonin receptor Mel_{1c} in the non-pigmented epithelium of the chicken (Wiechmann & Wirsig-Wiechmann, 2001) suggests that melatonin may affect the rate of aqueous humor secretion by the ciliary epithelium and the circadian rhythm of IOP.

The Mel_{1c} receptor subtype was first cloned in the chicken (Liu et al., 1995) and has never been identified in mammals (Wiechmann & Summers, 2008). The three identified melatonin receptors, namely MT1, MT2 and Mel_{1c}, show different daily rhythms of protein expression in the retinal pigment epithelium (RPE) and choroid, with peak levels of MT1 and MT2 occurring during the night and peak levels of Mel_{1c} occurring during the day in the chick (Rada & Wiechmann, 2006).

The presence of MT2 in *Xenopus* apical microvillar cell membrane but not on the basement membrane of the RPE supports the hypothesis that melatonin is involved in photoreceptor outer segment disk shedding and phagocytosis (Wiechmann & Rada, 2003; Wiechmann & Summers, 2008). It was originally reported that in the human retina, MT2 receptors are highly expressed compared to a relatively lower expression of MT1 receptors (Reppert et al., 1995), however a subsequent study reported similar levels of expression for both melatonin receptor types (Scher et al., 2002). As shown in Figure 1, MT1 receptors have been identified in RGCs and amacrine cells in rat and guinea pig (Fujieda & Hamadanizadeh, 1999; Fujieda et al., 2000). MT1 receptors are expressed in photoreceptors and other cell types of the human retina (Meyer et al., 2002; Scher et al., 2002, 2003). In humans, MT1 immunoreactivity was found in cell bodies along inner border of the inner nuclear layer, and in its outer border almost exclusively in horizontal cells, in cell bodies within the ganglion cell layer and in the inner segments of rod photoreceptors (Scher et al., 2002). In addition, about two thirds of CA1 and CA2 dopaminergic neurons exhibited MT1 immunolabeling. MT1 receptors were also identified in human and macaque AII amacrine cells, which are critical neurons in the rod pathway of the mammalian retina.

The MT2 receptor is expressed in the sclera, lens, RPE and neural retina of *Xenopus* eye (Wiechmann et al., 2004). In elderly humans, MT2 has recently been localized to ganglion and bipolar cells in the inner nuclear layer, to the inner segments of photoreceptors, and to cellular processes in inner and outer plexiform layers (Savaskan et al., 2007). The presence of melatonin receptors in multiple cell types suggests that melatonin could have multiple physiological functions in the retina (Figure 1). In human RGCs, MT1 receptors represent nearly 90% of the total number of melatonin receptors (Meyer et al., 2002). In one study, the MT1 receptor subtype expression in ganglion and amacrine cells from two patients with Alzheimer's disease was found to be significantly higher than in controls (Savaskan et al., 2002a), suggesting that in this disease an up-regulation mechanism exists that evolves with very low melatonin levels (Ferrari et al., 2000). The MT1 receptor has been identified in the adventitial cells of retinal vessels, suggesting that melatonin could have an indirect action on vascular smooth muscle (Savaskan et al., 2002b).

Based on these studies, it is now well established that melatonin receptors are present in the human eye, despite the lack of apparent melatonin synthesis in this structure. The MT1 and MT2 receptors are responsive to N-acetylserotonin albeit with a considerably lower sensitivity, thus N-actylserotonin which changes rapidly in response to light and dark signals might substitute for melatonin. More importantly, circulating melatonin can interact with these receptors.

4. Ocular functions of melatonin

Systemic administration of melatonin has been found to produce significant changes in anterior and vitreous chamber depth, suggesting that melatonin may play a role in ocular growth and development (Rada & Wiechmann, 2006). Retinal melatonin acts as a neuromodulator that mediates dark adaptive regulation of retinomotor movements (Pierce & Besharse, 1985). The expression of MT1 receptors in most dopaminergic amacrine cells in human retina implicates melatonin in the modulation of dopaminergic function (Scher et al., 2003). Both dopamine and melatonin are key signaling agents in the regulation of retinal rhythmicity. These two substances are mutually inhibitory, acting as signals for day and night, respectively. It was demonstrated that picomolar concentrations of melatonin selectively inhibit the calcium-dependent release of dopamine from rabbit retina (Dubocovich, 1983). Moreover, experimental evidence which supports the model of mutual signaling between melatonin and dopamine has been provided by Doyle et al. (Doyle et al., 2002)., who demonstrated that in C3H+/+ mice, which lack melatonin and show no circadian rhythmicity of dopamine content, the deficiency could be corrected by cyclical administration of melatonin. The action of melatonin on the rod pathway at the level of horizontal and amacrine cells has been proposed as a unique mechanism by which the retina adapts to low light intensities (Scher et al., 2002). A correlation between melatonin levels and the electroretinographic (ERG) response has been shown in human studies which suggest that daily melatonin and ERG cycling are associated (Rufiange et al., 2002). On the other hand, melatonin decreases retinal cAMP accumulation (Faillace et al., 1994) and regulates the activity of the retinal glutamate/glutamine cycle activity (Sáenz et al., 2004).

5. Oxidative and nitrosative stress in the eye and the role of melatonin as an antioxidant and antinitridergic agent

An increasing amount of evidence indicates that free radical generation is a major contributing factor to various eye diseases and that melatonin is an important ocular antioxidant (Siu et al., 2006; Lundmark et al., 2006). Eye exposure to intense illumination from focal light (Dorey et al., 1990; Cruickshanks et al., 1993), the presence of high oxygen tension in the eye (Alder & Cringle, 1985), free radicals generated from phagocytosis and lipid peroxidation (Tate, Jr. et al., 1995) as well as age-related oxidation (Cai et al., 2000; Militante & Lombardini, 2004) contribute to increased oxidative stress. Exposure of rod photoreceptor outer segments to bright light of 485 nm induces oxidation followed by irreversible damage (Marchiafava & Longoni, 1999). Oxidative stress has been proposed as a primary cause for retinopathy in preterm infants since it is associated with low levels of reduced glutathione (Papp et al., 1999) and vitamin E (Chan et al., 1999). Additionally, several lines of evidence support that retinal oxidative damage also plays a significant role in other ophthalmic diseases such as diabetic retinopathy (van Reyk et al., 2003; Yulek et al., 2007), retinal ischemia (Yilmaz et al., 2002; Aydemir et al., 2004), glaucoma (Moreno et al. 2004), and uveitis (Demir et al., 2006), among others.

UV-B radiation augments free radical production in corneal epithelial cells resulting in keratitis, whereas melatonin protects corneal epithelial cells from oxidative damage caused by exposure to UV radiation (Ciuffi et al., 2003).

The possibility that melatonin could detoxify highly reactive oxygen species was originally suggested by Ianas et al. (Ianas et al., 1991). Three years later, Reiter and coworkers (Reiter et al., 1994) using spin trapping and electron resonance spectroscopy, demonstrated melatonin's capacity to directly scavenge highly reactive hydroxyl radicals. Since then, several reports have shown that melatonin acts as a free radical scavenger and an efficient antioxidant (Hardeland et al., 1995; Reiter et al., 1997; Reiter et al., 1998; Reiter et al., 2000; Pandi-Perumal et al., 2006). Not only melatonin, but also several of its metabolites generated during its free radical scavenging action act as antioxidants (Tan et al., 2007a). The kynurenic pathway of melatonin

metabolism includes a series of radical scavengers with the possible sequence: Melatonin → cyclic 3-hydroxymelatonin → N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) → N^1-acetyl-5-methoxykynuramine (AMK). In the metabolic step from melatonin to AFMK, up to four free radicals can be consumed (Adler et al., 1997; Guenther et al., 2005; Hardeland, 2005; Tan et al., 2007a). Because of this pathway, melatonin's efficacy as an antioxidant is greatly increased. Melatonin has been shown to scavenge free radicals generated in mitochondria, reduce electron leakage from the respiratory complexes and improve ATP synthesis (Acuña-Castroviejo et al., 2003; Leon et al., 2005). Moreover, melatonin preserves mitochondrial glutathione levels, thereby enhancing the antioxidant potential (Leon et al., 2004). In addition, it was demonstrated that melatonin inhibits the nitridergic pathway activity in the golden hamster retina (Sáenz et al., 2002). In this study, melatonin was found to significantly decrease retinal NOS activity and L-arginine uptake, and additionally to inhibit the accumulation of cGMP induced by both L-arginine and a NO donor. The inhibitory effect of melatonin on retinal NOS activity is consistent with the previously described effect of melatonin on this enzyme from other neural structures (Bettahi et al., 1996; Pozo et al., 1997; Leon et al., 1998). However, while the effect of melatonin in those tissues was evident up to 1 nM, a much higher sensitivity to the methoxyindole was evident in the hamster retina, since it is effective even at 1 pM, suggesting that the retinal nitridergic pathway is regulated by physiological concentrations of melatonin (Sáenz et al., 2002). In addition to inhibiting NOS activity, melatonin is able to directly scavenge NO, generating al least one stable product, i.e., N-nitrosomelatonin (Turjanski et al., 2000). By scavenging free radicals, increasing the antioxidant defense system activity, and improving the electron transport chain at the mitochondrial level, melatonin is able to protect ocular tissues from both oxidative and nitrosative damage (Siu et al., 2006; Lundmark et al., 2006).

Because the retina in humans is unlikely to synthesize melatonin it is important to consider the possible role of its precursor N-acetylserotonin as an antioxidant. Numerous studies have now established that N-acetylserotonin has major antioxidant effects. Its mechanism of action and range of effects appears to be similar to melatonin and it has also been reported to be more potent than melatonin in several test systems (Perianayagam et al., 2005; Oxenkrug, 2005; Leaden & Catala, 2007; Gesing & Karbownik-Lewinska, 2008). Moreover, it has recently been shown that N-acetylserotonin decreases glutamate-induced lipid peroxidation in porcine retinal homogenates (Tang et al., 2007).

It was recently demonstrated that the low affinity MT3 melatonin receptor binding site is identical with quinone reductase 2 (QR2) (Nosjean et al., 2000), and that melatonin inhibits the activity of this enzyme (Boutin et al., 2008; Calamini et al., 2008) and there is evidence that, contrary to previous belief (Tan et al., 2007b), QR2 is an activating enzyme (Long et al., 2002; Celli et al., 2006) since its deletion from living organisms leads to increased toxicity of quinones (Long et al., 2002). Thus, inhibition of the enzyme could have antioxidant effects. Moreover, N-acetylserotonin interacts with QR2 and in fact has an affinity for this enzyme that is higher than that of melatonin (Molinari et al., 1996; Mailliet et al., 2005; Calamini et al., 2008). Therefore, endogenous levels of N-acetylserotonin are even more inhibitory to QR2 in producing an antioxidant action and may well be the endogenous ligand for this enzyme.

6. Melatonin and Uveitis

Uveitis is a common form of intraocular inflammation which is characterized by the disruption of the BOB, an event that provokes protein leakage and leukocyte infiltration into the aqueous humor. Uveitis is a disease that may produce blindness, i.e. among its sequellae are synequiae, cataract, and macular and optic nerve edema, all frequently associated with loss of vision. Current treatments for uveitis have limited effectiveness and a considerable range of side effects.

In recent studies, Kukner and coworkers (Kukner et al., 2006) and Sande and collaborators (Sande et al., 2008) investigated the effectiveness of melatonin as a therapeutic strategy in uveitis. In the Kukner study, intravitreal injections of bovine serum albumin were used to induce uveitis in the guinea pig. Melatonin, vitamin E, or aprotinin were intraperitoneally administered three days after albumin injection. A significant increase in leptin expression occurs in the retina, choroid, sclera, and episclera; ganglion cells were edematous and inner plexiform layer thickness increased in uveitic eyes. In animals treated with melatonin, vitamin E, or aprotinin, leptin expression was similar to the control group and edema and histopathological changes were reduced. Sande et al. studied endotoxin-induced uveitis, another established experimental model for uveitis, giving a single intravitreal injection of bacterial lipopolysaccharide (LPS) in the golden hamster. LPS, a component of Gram-negative bacterial outer membranes, enhances the expression of various inflammatory mediators including tumor necrosis factor (TNF)-α, interleukin (IL)-6 (de Vos et al., 1994), prostaglandin E_2 (Bellot et al., 1996), and NO (Nathan & Xie, 1994; Jacquemin et al., 1996; Hashida et al., 2000). In this study, a pellet of melatonin was implanted subcutaneously two hours before the intravitreal injection of LPS. Twenty-four hours and eight days after injection, the inflammatory response was evaluated in terms of: i) integrity of BOB blood-ocular barrier, ii) clinical signs, iii) histopathological studies, and iv) retinal function. Melatonin reduced the leakage of proteins and cells in the anterior segment of LPS-injected eyes, decreased clinical signs, and protected the ultrastructure of blood-ocular barriers. In animals injected with LPS, a remarkable disorganization of rod outer segment membranous disks was observed, whereas no morphological changes in photoreceptor outer segments were observed in animals treated with LPS plus melatonin. Furthermore, melatonin prevented the decrease in the electroretinographic activity induced by LPS. Melatonin significantly abrogated the LPS-induced increase in retinal NOS activity, TNFα, and nuclear factor κB (NFκB) p50 and p65 subunits levels. These results indicate that melatonin prevents clinical, biochemical, histological, ultrastructural, and functional consequences of experimental uveitis, probably through an NFκB-dependent mechanism. Both groups concluded that their findings justified further investigations of melatonin's use as a new therapeutic strategy for the treatment of uveitis.

7. Melatonin and glaucoma

Glaucoma, a chronic disease characterized by visual field loss, cupping of the optic nerve head, and irreversible loss of RGCs, is a leading cause of blindness worldwide. It is estimated that half of those affected may not be aware of their condition because symptoms may not occur during the early stages of the disease. When vision loss appears, considerable and permanent damage has already occurred. Medications and surgery can help slow the progression of some forms of the disease, but there is no cure at present. Increased IOP is considered the major risk factor in glaucoma, but visual field loss may continue despite successful lowering IOP. Although the clinical features of glaucoma are well described, the mechanisms resulting in optic nerve damage and RGC death remain to be elucidated.

Melatonin concentration in aqueous humor parallels its concentration in plasma, peaking during the dark period (Yu et al., 1990; Liu & Dacus, 1991). Inasmuch as the circadian rhythm of aqueous humor secretion likely contributes to the circadian rhythm of IOP (Smith & Gregory, 1989), it has been suggested that melatonin affects aqueous humor secretion (Wiechmann & Wirsig-Wiechmann, 2001). In nocturnal animals, IOP is low during the light period and high in the dark period (Frampton et al., 1987; Smith & Gregory, 1989; Liu & Dacus, 1991; Benozzi et al., 2002). In contrast, studies in diurnally active humans reveal that IOP levels peak during

daytime (Kitazawa & Horie, 1975). Although it has been demonstrated that the administration of melatonin reduces IOP in humans (Samples et al., 1988), to what extent melatonin levels affect circadian IOP rhythm remains to be defined (Osborne, 1994; Pintor et al., 2001).

The effect on IOP of topical application of the MT3 (QR2) melatonin binding site agonist 5-methoxycarbonylamino-N- acetyltryptamine (5-MCA-NAT) was evaluated in glaucomatous monkey's eye (Serle et al., 2004). It was demonstrated that 5-MCA-NAT substantially reduces IOP in glaucomatous monkeys with reductions in IOP ranging from 10 % on day one up to 19 % on day 5, the ocular hypotensive effect lasting for at least 18 hours.

In addition to ocular hypertension, several concomitant factors including elevation of glutamate levels, disorganized NO metabolism, and oxidative damage caused by overproduction of ROS, may significantly contribute to glaucomatous neurodegeneration [for a review, see Hashida et al., 2000). In particular, NO is believed to play a significant role in experimental glaucoma (Neufeld et al., 1997; Neufeld, 2004; Belforte et al., 2007). Although NO is a ubiquitous signaling molecule that participates in a variety of cellular functions, in concert with reactive oxygen species, NO can be transformed into a highly potent and effective cytotoxic entity with pathophysiological significance. NO may also signal through the interaction with reduced cysteines of proteins, changing their function (Martinez-Ruiz & Lamas, 2004), and it modulates the activity of various proteins that contribute to apoptosis (Melino et al., 1997). Furthermore, it has been demonstrated that an extracellular proteolytic pathway in the retina contributes to retinal ganglion cells death *via* NO-activated metalloproteinase-9 (Manabe et al., 2005).

Several studies, most of them based on Western blotting or immunohistochemical analysis, addressed the issue of NO involvement in human or experimental glaucoma. Increased levels of neuronal NOS (NOS-1) and inducible NOS (NOS-2) were reported in astrocytes of the lamina cribosa and optic nerve head from patients with primary open-angle glaucoma (Liu & Neufeld, 2000). In rats whose extraocular veins were cauterized to produce chronic ocular hypertension and retinal damage, increased expression of NOS-2 but not NOS-1 was found in optic nerve head astrocytes (Wang et al., 2005). Moreover, elevation of hydrostatic pressure *in vitro* was associated with upregulation of NOS-2 expression in human astrocytes derived from the optic nerve head (Liu & Neufeld, 2000). Most importantly, inhibition of NOS-2 was found to protect against ganglion cell loss in the rat cautery model of glaucoma (Neufeld, 2004). These data support that activation of NOS, especially NOS-2, may play a significant role in glaucomatous optic neuropathy. However, in contrast to these results, Pang et al. (Pang et al., 2005) showed that chronically elevated IOP in the rat induced by episcleral injection of hypertonic saline does not increase NOS-2 immunoreactivity in the optic nerve head, nor in ganglion cell layer. Moreover, retinal and optic nerve head NOS-2 mRNA levels did not correlate with either IOP level or severity of optic nerve injury, and, additionally, there was no difference between glaucomatous and non-glaucomatous eyes in terms of NOS-2 immunoreactivity in the optic nerve head. Furthermore, aminoguanidine treatment did not affect the development of pressure-induced optic neuropathy in rats (Pang et al., 2005). As already mentioned, these studies did not assess changes in the functional capacity of the retinal nitridergic pathway. More recently, in another experimental model of glaucoma (induced by intracameral injections of hyaluronic acid (HA)) a significant activation of retinal nitridergic pathway was demonstrated (Belforte et al., 2007). In this study, it was shown that retinal NOS activity significantly increases in hypertensive eyes, although no changes in the levels of NOS isoforms were observed in HA-treated eyes. Different mechanisms might modulate NOS activity, including changes in substrate supply, protein phosphorylation, and subcellular localization, among others. The intracellular events triggered by ocular hypertension that could explain the association between ocular hypertension and NOS activity, as well as the isoform(s) of NOS whose activity is augmented by HA-induced ocular hypertension, remain to be defined. However, since glutamate activity at

NMDA receptors is one of the most conspicuous promoters of NOS-1 activity, the increase in glutamate synaptic levels previously demonstrated in HA-treated eyes (Moreno et al., 2005) could account for an increase in nNOS activity in this experimental model. Thus, the activation of NOS in hypertensive eyes can be linked to glutamate levels that, in turn, might be elevated to such an extent that they are toxic for ganglion cells. Also in this regard, it has been shown that RGCs in the nNOS-deficient mouse are fairly resistant to NMDA, while damage in the retina of the eNOS-deficient mouse is not distinguishable from that observed in control animals (Vorwerk et al., 1997).

In addition to NOS activity, another limiting step in the regulation of NO biosynthesis is the availability of the precursor L-arginine. L-arginine influx and mRNA levels of cationic amino acid transporter type 1 and 2 (CAT-1 and CAT-2) are significantly increased in the retinas from hypertensive eyes (Belforte et al., 2007). Purified NOS from different sources has been reported to have a low half-saturating L-arginine concentration (EC_{50} ~ 10 μM). Since high levels of intracellular L-arginine ranging from 0.1 - 1 mM have been measured in many systems (Block et al., 1995), it is expectable that endogenous L-arginine would support maximal activation of NOS. However, several *in vivo* and *in vitro* studies indicate that NO production under physiological conditions can be enhanced by extracellular L-arginine, despite saturating intracellular L-arginine concentrations. This has been termed "the arginine paradox" (Kurz & Harrison, 1997). One possible explanation could be that intracellular L-arginine is sequestered in one or more pools that are poorly, if at all, accessible to NOS, whereas extracellular L-arginine transported into the cells is preferentially delivered to NO biosynthesis (Kurz & Harrison, 1997). Therefore, it seems likely that to induce the activation of NOS, an influx of L-arginine is essential. The coordination between NOS activity and L-arginine uptake has been demonstrated in several systems such as diabetic rat retina (do Carmo et al., 1998). A similar coordination between NO biosynthesis and intracellular L-arginine availability seems to occur in the retina from hypertensive eyes. Recently, it was demonstrated that activation of NMDA receptors in cultured retinal cells promoted an increase in the intracellular L-arginine pool available for NO synthesis (Cossenza et al., 2006). In this process, the increase in both NOS activity and L-arginine influx could be triggered by higher levels of synaptic glutamate levels in retinas from eyes injected with HA (Moreno et al., 2005). Notably, it was demonstrated that both diurnal and nocturnal retinal melatonin levels decreased in hypertensive eyes (Moreno et al., 2004). In addition, a significant decrease in the retinal antioxidant defense system activity was observed in the retinas from eyes injected with HA. Taking into account the conclusive evidence from numerous studies showing that melatonin has significant antioxidant and antinitridergic activity, together with the correlative evidence that retinal melatonin levels are reduced in tandem with the decrease in the antioxidant defense system activity and the increase in the retinal nitridergic pathway, it is tempting to postulate that a causal relationship exists between these phenomena.

Defected mitochondrial respiratory chain, in addition to causing a severe ATP deficiency, often augments ROS generation in mitochondria, which enhances pathological conditions and diseases. In fact, mitochondrial dysfunction-associated oxidative stress has also been implicated as a risk factor in glaucoma patients (Abu-Amero et al., 2006). In this sense, it was shown that melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production (López et al, 2009). Moreover, it was demonstrated that melatonin inhibits mitochondrial NOS isoform (Escames et al., 2007).

In the glaucoma model induced by HA injections, the retinal nitridergic pathway activation and the decrease in the antioxidant defense system preceded both functional and histological alterations provoked by ocular hypertension. Therefore, it is possible that oxidative stress and an overactivation of the retinal nitridergic system could contribute to the hypertension-induced retinal damage. In the glaucoma model induced by chronic injections of HA, it has been recently

shown that a subcutaneous pellet of melatonin significantly prevents the electroretinographic dysfunction and diminishes the vulnerability of retinal ganglion cells to the deleterious effects of ocular hypertension (Rosenstein et al., 2009).

Besides the effect of melatonin as a retinal antioxidant which could protect retinal ganglion cells from ocular hypertensive damage, the pathogenic role of oxidative stress in increasing IOP by reducing aqueous outflow facility is supported by various experimental studies performed *in vitro* and *in vivo*. *In vitro* treatment of human trabecular meshwork cells with hydrogen peroxide alters cellular adhesion and integrity (Zhou et al, 1999). In an animal study, perfusion of trabecular meshwork cells with peroxide has shown to reduce aqueous humor drainage from the anterior chamber of the calf's eye (Kahn et al., 1983). Moreover, human trabecular meshwork endothelium has been reported to be an enriched site of NO synthesis. NO can interact with oxygen or metals, such as copper or iron, to modulate outflow resistance of the trabecular meshwork (Haefliger et al., 1999). In this way, being an effective antioxidant and an antinitridergic melatonin can be beneficial not only at retinal level, but also in the eye anterior chamber, contributing to restore the aqueous humor drainage.

Besides the mechanisms already described, there are other beneficial mechanisms of melatonin for glaucoma treatment (Figure 2). Several lines of evidence support that the obstruction of retrograde transport at the optic nerve head results in the deprivation of neurotrophic support to RGCs, leading to apoptotic cell death in glaucoma (Quigley et al., 2000; Johnson et al., 2009). An important corollary to this concept is the implication that appropriate enhancement of neurotrophic support will prolong the survival of injured RGC. Of particular importance is the fact that brain-derived neurotrophic factor (BDNF) not only promotes ganglion cell survival following damage to the optic nerve, but also helps to preserve the structural integrity of the surviving neurons, which in turn results in enhanced visual function (Weber et al., 2008). As for the link between melatonin and neurotrophins, it has been suggested that melatonin may participate in neurodevelopment and in the regulation of neurotrophic factors (Jimenez et al., 2007; Niles et al., 2004). *In vitro*, melatonin promotes the viability and neuronal differentiation of neural stem cells and increases their production of BDNF (Kong et al., 2008). Moreover, ramelteon (a melatonin receptor agonist) is capable of increasing BDNF protein in primary cultures of cerebellar granule cells (Imbesi et al., 2008).

In addition to ocular hypertension, the majority of glaucoma patients show signs of reduced ocular blood flow as well as ischemic signs in the eye, supporting that hemodynamic factors are involved as well in glaucomatous neuropathy. In this sense, it was shown that melatonin could increase the survival rate and rescue and restore injured RGCs in an experimental model of ischemia/reperfusion in rats (Tang et al., 2006), and it counteracts ischemia-induced apoptosis in human retinal pigment epithelial cells (Osborne et al., 1998).

Finally, while the cellular mechanisms involved in the loss of ganglion cells observed in glaucomatous neuropathy are based on a phenomenon of apoptosis, melatonin was shown to have antiapoptotic properties acting through several mechanisms, such as reduction of caspases, cytochrome c release, and modulation of Bcl-2 and Bax genes, among others. Figure 2 summarizes some of the ethiopathogenic mechanisms involved in glaucomatous neuropathy and the effect of melatonin on these mechanisms.

8. Neuroprotective drugs in the treatment of glaucoma

According to Osborne et al. (Osborne et al., 1999), neuroprotective agents will be more beneficial to patients in which neurons die slowly, as seen in glaucoma, than in a disease in which the death of a set of neurons is more rapid. Many compounds such as betaxolol,

brimonidine, calcium channel blockers, antioxidants such as vitamin E and coenzyme Q, and *Ginkgo biloba* extracts have been tried in animals and have been shown to protect the retina against free radical damage and lipid peroxidation (Ritch, 2000). Calcium channel blockers have been shown to neutralize NMDA-induced intracellular Ca^{2+} influx. Netland and coworkers (Netland et al., 1993) demonstrated a decrease in glaucoma progression in patients treated with Ca^{2+} channel blockers. On the other hand, the NMDA antagonist memantine effectively blocked the excitotoxic response of RGCs both in culture and *in vivo* conditions (Vorwerk et al., 1996). However, a recent study showed that the progression of glaucoma was significantly lower in patients receiving a higher dose of memantine than in patients receiving a low dose of memantine, but there was no clear benefit compared to patients receiving placebo.

Melatonin has been demonstrated to be an effective neuroprotective agent in various experimental models and also is being used in the treatment of neurodegenerative diseases such as Alzheimer disease and Parkinsonism, where it has been shown to improve the clinical condition of the patients (Reiter et al., 1999; Srinivasan et al., 2005, 2006; Furio et al., 2007; Dowling et al., 2008). As discussed above, melatonin acts as an efficient retinal antioxidant (Siu et al., 1999). In addition, melatonin has been shown to act as a potent inhibitor of the retinal nitridergic pathway since it directly reacts with NO (Turjanski et al., 2000), decreases NOS activity, the uptake of NOS substrate (L-arginine), as well as the increase in cGMP content induced by L-arginine (Sáenz et al., 2002). Moreover, melatonin is a potent inhibitor of NOS-1 and NOS-2 gene expression (Poliandri et al., 2006) and it also reduces NO-induced retinal oxidative damage both *in vitro* (Siu et al., 1999) and *in vivo* (Siu et al., 2004). In view of this evidence, melatonin could be a promising agent for the management of glaucoma, inasmuch as it exhibits antioxidant and antinitridergic properties, as well as reducing retinal glutamate synaptic levels, among other mechanism (Figure 2).

As already mentioned, the current management of glaucoma is mainly directed at the control of IOP. However, it would be clearly preferable for a therapy to have as its main goal the prevention of the death of ganglion cells rather than a symptomatic treatment. The results presented above support the conclusion that a decrease in the retinal nitridergic pathway activity as well as an antioxidant treatment may prevent glaucomatous cell death. Melatonin, by itself, may fulfill all these requirements and thus, therapies based on the application of melatonin may have significant potential as a new strategy in glaucoma management.

10. Concluding remarks

Uveitis and glaucoma are highly prevalent ocular diseases, and constitute main causes of blindness. Thus, they represent a significant public health concern. Uveitis is essentially an inflammatory disease, while glaucoma is an ocular dysfunction highly associated with ocular hypertension and characterized by a loss of RGCs and optic nerve head atrophy. Uveitis and glaucoma differ in several aspects, including causes, risk factors, and retinal cell types involved, among many others. However, several lines of evidence support the inference that uveitis and glaucoma may share some of the ethiopathogenic mechanisms, particularly oxidative damage and increased NO production. Indeed, there is experimental evidence supporting the value of antioxidant and antinitridergic compounds for treating both glaucoma and uveitis (Aydemir et al., 2004; Kukner et al., 2006). As already mentioned, melatonin by itself exhibits both antioxidant and antinitridergic properties. An effective neuroprotectant for glaucoma treatment must reach the optic nerve head and/or ganglion cells and will therefore probably have to be taken orally, and because it will reach other parts of the body, any side-effect of an appropriate neuroprotectant must be reduced to a minimum (Osborne, 2009). Melatonin, a very safe compound for human use, is highly lipophilic and readily diffuses into tissues. The results

presented herein support that alone or combined with an ocular hypotensive therapy (in glaucoma) or with corticosteroid (in uveitis), melatonin should be included in the armamentarium of ophthalmic therapeutic resources, particularly for the treatment of uveitis and glaucoma.

REFERENCES

Abe, M., Itoh, M.T., Miyata, M., Ishikawa, S., Sumi,Y., 1999. Detection of melatonin, its precursors and related enzyme activities in rabbit lens. Exp. Eye Res. 68, 255-262.

Abe, M., Itoh, M.T., Miyata, M., Shimizu, K., Sumi,Y., 2000. Circadian rhythm of serotonin N - acetyltransferase activity in rat lens. Exp. Eye Res. 70, 805-808.

Abu-Amero, K.K,, Morales, J., Bosley, T.M., 2006. Mitochondrial abnormalities in patients with primary open-angle glaucoma. Invest. Ophthalmol. Vis. Sci. 47, 2533-2541.

Acuña-Castroviejo, D., Escames, G., Leon, J., Carazo, A., Khaldy, H., 2003. Mitochondrial regulation by melatonin and its metabolites. Adv. Exp. Med. Biol. 527, 549-557.

Adler, L.J., Gyulai, F.E., Diehl, D.J., Mintun, M.A., Winter, P.M., Firestone, L.L., 1997. Regional brain activity changes associated with fentanyl analgesia elucidated by positron emission tomography. Anesth. Analg. 84, 120-126.

Alarma-Estrany, P., Pintor, J., 2007. Melatonin receptors in the eye: location, second messengers and role in ocular physiology. Pharmacol. Ther. 113, 507-522.

Alder,V.A., Cringle, S.J., 1985. The effect of the retinal circulation on vitreal oxygen tension. Curr. Eye Res. 4, 121-129.

Alonso-Gomez, A.L., Iuvone, P.M., 1995. Melatonin biosynthesis in cultured chick retinal photoreceptor cells: calcium and cyclic AMP protect serotonin N-acetyltransferase from inactivation in cycloheximide-treated cells. J. Neurochem. 65, 1054-1060.

Ascher, J.A., Cole, J.O., Colin, J.N., Feighner, J.P., Ferris, R.M., Fibiger, H.C., Golden, R.N., Martin, P., Potter, W.Z., Richelson, E., 1995. Bupropion: a review of its mechanism of antidepressant activity. J.Clin. Psychiatry 56, 395-401.

Axelrod, J., 1974. The pineal gland: a neurochemical transducer. Science 184, 1341-1348.

Aydemir, O., Celebi, S., Yilmaz, T., Yekeler, H., Kukner, A.S., 2004. Protective effects of vitamin E forms (alpha-tocopherol, gamma-tocopherol and d-alpha-tocopherol polyethylene glycol 1000 succinate) on retinal edema during ischemia-reperfusion injury in the guinea pig retina. Int. Ophthalmol. 25, 283-289.

Bailey, M.J., Chong, N.W., Xiong, J., Cassone, V.M., 2002. Chickens' Cry2: molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. FEBS Lett. 513, 169-174.

Belforte, N., Moreno, M.C., Cymeryng, C., Bordone, M., Keller Sarmiento, M.I., Rosenstein, R.E., 2007. Effect of ocular hypertension on retinal nitridergic pathway activity. Invest. Ophthalmol. Vis. Sci. 48, 2127-2133.

Bellot, J.L., Palmero, M., Garcia-Cabanes, C., Espi, R., Hariton, C., Orts, A., 1996. Additive effect of nitric oxide and prostaglandin-E2 synthesis inhibitors in endotoxin-induced uveitis in the rabbit. Inflamm. Res. 45, 203-208.

Benozzi, J., Nahum, L.P., Campanelli, J.L., Rosenstein, R.E., 2002. Effect of hyaluronic acid on intraocular pressure in rats. Invest. Ophthalmol. Vis. Sci. 43, 2196-2200.

Bernard, M., Donohue, S.J., Klein, D.C., 1995. Human hydroxyindole-O-methyltransferase in pineal gland, retina and Y79 retinoblastoma cells. Brain Res. 696, 37-48.

Bernard, M., Guerlotte, J., Greve, P., Grechez-Cassiau, A., Iuvone, M.P., Zatz, M., Chong, N.W., Klein, D.C., Voisin, P., 1999. Melatonin synthesis pathway: circadian regulation of the genes encoding the key enzymes in the chicken pineal gland and retina. Reprod. Nutr. Dev. 39, 325-334.

Bettahi, I., Pozo, D., Osuna, C., Reiter, R.J., Acuña-Castroviejo, D., Guerrero, J.M., 1996. Melatonin reduces nitric oxide synthase activity in rat hypothalamus. J. Pineal Res. 20, 205-210.

Block, E.R., Herrera, H., Couch, M., 1995. Hypoxia inhibits L-arginine uptake by pulmonary artery endothelial cells. Am. J. Physiol. 269, L574-L580.

Boutin, J.A., Audinot, V., Ferry, G., Delagrange, P., 2005. Molecular tools to study melatonin pathways and actions. Trends Pharmacol. Sci. 26, 412-419.

Boutin, J.A., Marcheteau, E., Hennig, P., Moulharat, N., Berger, S., Delagrange, P., Bouchet, J.P., Ferry, G., 2008. MT3/QR2 melatonin binding site does not use melatonin as a substrate or a co-substrate. J. Pineal Res. 45, 524-531.

Bunin, A.I., Filina, A.A., Erichev, V.P., 1992. [A glutathione deficiency in open-angle glaucoma and the approaches to its correction]. Vestn. Oftalmol. 108, 13-15.

Cahill, G.M., Besharse, J.C., 1993. Circadian clock functions localized in xenopus retinal photoreceptors. Neuron 10, 573-577.

Cai, J., Nelson, K.C., Wu, M., Sternberg, P. Jr., Jones, D.P., 2000. Oxidative damage and protection of the RPE. Prog. Retin. Eye Res. 19, 205-221.

Calamini, B., Santarsiero, B.D., Boutin, J.A., Mesecar, A.D., 2008. Kinetic, thermodynamic and X-ray structural insights into the interaction of melatonin and analogues with quinone reductase 2. Biochem. J. 413, 81-91.

Cardinali, D.P., Rosner, J.M., 1971a. Metabolism of serotonin by the rat retina in vitro. J. Neurochem. 18, 1769-1770.

Cardinali, D.P., Rosner, J.M., 1971b. Retinal localization of the hydroxyindole-0-methyl transferase (HIOMT) in the rat. Endocrinology 89, 301-303.

Celli, C.M., Tran, N., Knox, R., Jaiswal, A.K., 2006. NRH:quinone oxidoreductase 2 (NQO2) catalyzes metabolic activation of quinones and anti-tumor drugs. Biochem. Pharmacol. 72, 366-376.

Chan, D.K., Lim, M.S., Choo, S.H., Tan, I.K., 1999. Vitamin E status of infants at birth. J. Perinat. Med. 27, 395-398.

Chomarat, P., Coge, F., Guenin, S.P., Mailliet, F., Vella, F., Mallet, C., Giraudet, S., Nagel, N., Leonce, S., Ferry, G., Delagrange, P., Boutin, J.A., 2007. Cellular knock-down of quinone reductase 2: a laborious road to successful inhibition by RNA interference. Biochimie 89, 1264-1275.

Ciuffi, M., Pisanello, M., Pagliai, G., Raimondi, L., Franchi-Micheli, S., Cantore, M., Mazzetti, L., Failli, P., 2003. Antioxidant protection in cultured corneal cells and whole corneas submitted to UV-B exposure. J. Photochem. Photobiol. B. 71, 59-68.

Coon, S.L., Mazuruk, K., Bernard, M., Roseboom, P.H., Klein, D.C., Rodriguez, I.R., 1996. The human serotonin N-acetyltransferase (EC 2.3.1.87) gene (AANAT): structure, chromosomal localization, and tissue expression. Genomics 34, 76-84.

Coon, S.L., Del, O.E., Young, W.S. III., Klein, D.C., 2002. Melatonin synthesis enzymes in Macaca mulatta: focus on arylalkylamine N-acetyltransferase (EC 2.3.1.87). J. Clin. Endocrinol. Metab. 87, 4699-4706.

Cossenza, M., Cadilhe, D.V., Coutinho, R.N., Paes-de-Carvalho, R., 2006. Inhibition of protein synthesis by activation of NMDA receptors in cultured retinal cells: a new mechanism for the regulation of nitric oxide production. J. Neurochem. 97, 1481-1493.

Cruickshanks, K.J., Klein, R., Klein, B.E., 1993. Sunlight and age-related macular degeneration. The Beaver Dam Eye Study. Arch.Ophthalmol. 111, 514-518.

Dawson, T.M., Steiner, J.P., Dawson, V.L., Dinerman, J.L., Uhl, G.R., Snyder, S.H., 1993. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc. Natl. Acad. Sci. U.S.A. 90, 9808-9812.

de Vos, A.F., Klaren, V.N., Kijlstra, A., 1994. Expression of multiple cytokines and IL-1RA in the uvea and retina during endotoxin-induced uveitis in the rat. Invest. Ophthalmol. Vis. Sci. 35, 3873-3883.

Demir, D., Yilmaz, T., Ilhan, N., Yekeler, H., Aydemir, O., Kukner, A.S., 2006. Protective role of alpha-tocopherol on retinal injury in experimental uveitis in guinea pigs. Pathophysiology 13, 75-79.

Dinet, V., Girard-Naud, N., Voisin, P., Bernard, M., 2006. Melatoninergic differentiation of retinal photoreceptors: activation of the chicken hydroxyindole-O-methyltransferase promoter requires a homeodomain-binding element that interacts with Otx2. Exp. Eye Res. 83, 276-290.

do Carmo, A., Lopes, C., Santos, M., Proenca, R., Cunha-Vaz, J., Carvalho, A.P., 1998. Nitric oxide synthase activity and L-arginine metabolism in the retinas from streptozotocin-induced diabetic rats. Gen. Pharmacol. 30, 319-324.

Dorey, C.K., Delori, F.C., Akeo, K., 1990. Growth of cultured RPE and endothelial cells is inhibited by blue light but not green or red light. Curr. Eye Res. 9, 549-559.

Dowling, G.A., Burr, R.L., Van Someren, E.J., Hubbard, E.M., Luxenberg, J.S., Mastick, J., Cooper, B.A., 2008. Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. J. Am. Geriatr. Soc. 56, 239-246.

Doyle, S.E., Grace, M.S., McIvor, W., Menaker, M., 2002. Circadian rhythms of dopamine in mouse retina: the role of melatonin. Vis. Neurosci. 19, 593-601.

Drexler, H., Zeiher, A.M., Meinzer, K., Just, H., 1991. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 338, 1546-1550.

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

Dubocovich, M.L., 1995. Melatonin receptors: are there multiple subtypes? Trends Pharmacol. Sci. 16, 50-56.

Escames, G., López, L.C., Ortiz, F., López, A., García, J.A,, Ros, E., Acuña-Castroviejo, D., 2007. Attenuation of cardiac mitochondrial dysfunction by melatonin in septic mice. FEBS J. 274, 2135-2147.

Faillace, M.P., Cutrera, R., Sarmiento, M.I., Rosenstein, R.E., 1995. Evidence for local synthesis of melatonin in golden hamster retina. Neuroreport 6, 2093-2095.

Faillace, M.P., Sarmiento, M.I., Siri, L.N., Rosenstein, R.E., 1994. Diurnal variations in cyclic AMP and melatonin content of golden hamster retina. J. Neurochem. 62, 1995-2000.

Ferrari, E., Arcaini, A., Gornati, R., Pelanconi, L., Cravello, L., Fioravanti, M., Solerte, S.B., Magri, F., 2000. Pineal and pituitary-adrenocortical function in physiological aging and in senile dementia. Exp.Gerontol. 35, 1239-1250.

Frampton, P., Da, R.D., Brown, B., 1987. Diurnal variation of intraocular pressure and the overriding effects of sleep. Am. J. Optom. Physiol. Opt. 64, 54-61.

Fujieda, H., Hamadanizadeh, S.A., Wankiewicz, E., Pang, S.F., Brown, G.M., 1999. Expression of mt1 melatonin receptor in rat retina: evidence for multiple cell targets for melatonin. Neuroscience 93, 793-799.

Fujieda, H., Scher, J., Hamadanizadeh, S.A., Wankiewicz, E., Pang, S.F., Brown, G.M., 2000. Dopaminergic and GABAergic amacrine cells are direct targets of melatonin: immunocytochemical study of mt1 melatonin receptor in guinea pig retina. Vis. Neurosci. 17, 63-70.

Fukuhara, C., Dirden, J.C., Tosini, G., 2001. Photic regulation of melatonin in rat retina and the role of proteasomal proteolysis. Neuroreport 12, 3833-3837.

Fukuhara, C., Liu, C., Ivanova, T.N., Chan, G.C., Storm, D.R., Iuvone, P.M., Tosini, G., 2004. Gating of the cAMP signaling cascade and melatonin synthesis by the circadian clock in mammalian retina. J. Neurosci. 24, 1803-1811.

Furio, A.M., Brusco, L.I., Cardinali, D.P., 2007. Possible therapeutic value of melatonin in mild cognitive impairment: a retrospective study. J. Pineal Res. 43, 404-409.

Gan, J., Alonso-Gomez, A.L., Avendano, G., Johnson, B., Iuvone, P.M., 1995. Melatonin biosynthesis in photoreceptor-enriched chick retinal cell cultures: role of cyclic AMP in the K(+)-evoked, Ca(2+)-dependent induction of serotonin N-acetyltransferase activity. Neurochem. Int. 27, 147-155.

Garbarino-Pico, E., Carpentieri, A.R., Contin, M.A., Sarmiento, M.I., Brocco, M.A., Panzetta, P., Rosenstein, R.E., Caputto, B.L., Guido, M.E., 2004. Retinal ganglion cells are autonomous circadian oscillators synthesizing N-acetylserotonin during the day. J. Biol. Chem. 279, 51172-51181.

Gesing, A., Karbownik-Lewinska, M., 2008. Protective effects of melatonin and N-acetylserotonin on aflatoxin B1-induced lipid peroxidation in rats. Cell Biochem. Funct. 26, 314-319.

Greve, P., Alonso-Gomez, A., Bernard, M., Ma, M., Haque, R., Klein, D.C., Iuvone, P.M., 1999. Serotonin N-acetyltransferase mRNA levels in photoreceptor-enriched chicken retinal cell cultures: elevation by cyclic AMP. J. Neurochem. 73, 1894-1900.

Guenther, A.L., Schmidt, S.I., Laatsch, H., Fotso, S., Ness, H., Ressmeyer, A.R., Poeggeler, B., Hardeland, R., 2005. Reactions of the melatonin metabolite AMK (N1-acetyl-5-methoxykynuramine) with reactive nitrogen species: formation of novel compounds, 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. J. Pineal Res. 39, 251-260.

Haefliger, I.O., Dettmann, E., Liu, R., Meyer, P., Prünte, C., Messerli, J., Flammer, J., 1999. Potential role of nitric oxide and endothelin in the pathogenesis of glaucoma. Surv. Ophthalmol. 43, S51-58.

Haque, R., Chaurasia, S.S., Wessel, J.H. III., Iuvone, P.M., 2002. Dual regulation of cryptochrome 1 mRNA expression in chicken retina by light and circadian oscillators. Neuroreport 13, 2247-2251.

Hardeland, R., 2005. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 27, 119-130.

Hardeland, R., Balzer, I., Poeggeler, B., Fuhrberg, B., Uria, H., Behrmann, G., Wolf, R., Meyer, T.J., Reiter, R.J., 1995. On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. J. Pineal Res. 18, 104-111.

Harmar, A.J., Hills, R.A., Rosser, E.M., Jones, M., Buneman, O.P., Dunbar, D.R., Greenhill, S.D., Hale, V.A., Sharman, J.L., Bonner, T.I., Catterall, W.A., Davenport, A.P., Delagrange, P., Dollery, C.T., Foord, S.M., Gutman, G.A., Laudet, V., Neubig, R.R., Ohlstein, E.H., Olsen, R.W., Peters, J., Pin, J.P., Ruffolo, R.R., Searls, D.B., Wright, M.W., Spedding, M., 2009. IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels. Nucleic Acids Res. 37, D680-D685.

Hashida, M., Fukushima, A., Zhang, J., Kodama, H., Ueno, H., 2000. Involvement of superoxide generated by polymorphonuclear leukocytes in endotoxin-induced uveitis. Graefes Arch.Clin. Exp. Ophthalmol. 238, 359-365.

Ianas, O., Olinescu, R., Badescu, I., 1991. Melatonin involvement in oxidative processes. Endocrinologie 29, 147-153.

Imbesi, M., Uz, T., Manev, H., 2008. Role of melatonin receptors in the effects of melatonin on BDNF and neuroprotection in mouse cerebellar neurons. J. Neural Transm. 115, 1495-1499.

Itoh, M.T., Takahashi, N., Abe, M., Shimizu, K., 2007. Expression and cellular localization of melatonin-synthesizing enzymes in the rat lens. J. Pineal Res. 42, 92-96.

Iuvone, P.M., Brown, A.D., Haque, R., Weller, J., Zawilska, J.B., Chaurasia, S.S., Ma, M., Klein, D.C., 2002. Retinal melatonin production: role of proteasomal proteolysis in circadian and photic control of arylalkylamine N-acetyltransferase. Invest. Ophthalmol. Vis. Sci. 43, 564-572.

Ivanova, T.N., Iuvone, P.M., 2003. Melatonin synthesis in retina: circadian regulation of arylalkylamine N-acetyltransferase activity in cultured photoreceptor cells of embryonic chicken retina. Brain Res. 973, 56-63.

Jacquemin, E., de Kozak, Y., Thillaye, B., Courtois, Y., Goureau, O., 1996. Expression of inducible nitric oxide synthase in the eye from endotoxin-induced uveitis rats. Invest. Ophthalmol.Vis. Sci. 37, 1187-1196.

Jimenez-Jorge, S., Guerrero, J.M., Jimenez-Caliani, J., Naranjo, M.C., Lardone, P.J., Carrillo-Vico, A., Osuna, C., Molinero, P., 2007. Evidence for melatonin synthesis in the rat brain during development, J. Pineal Res. 42, 240–246.

Johnson, E.C., Guo, Y., Cepurna, W.O., Morrison, J.C., 2009. Neurotrophin roles in retinal ganglion cell survival: lessons from rat glaucoma models. Exp. Eye Res. 88, 808-815.

Kahn, M.G., Giblin, F.J., Epstein, D.L., 1983. Glutathione in calf trabecular meshwork and its relation to aqueous humor outflow facility. Invest. Ophthalmol. Vis. Sci. 24, 1283-1287.

Kaushik, S., Pandav, S.S., Ram, J., 2003. Neuroprotection in glaucoma. J. Postgrad. Med. 49, 90-95.

Kitazawa,Y., Horie, T., 1975. Diurnal variation of intraocular pressure in primary open-angle glaucoma. Am. J. Ophthalmol. 79, 557-566.

Kong, X., Li, X., Cai, Z., Yang, N., Liu, Y., Shu, J., Pan, L., Zuo, P., 2008. Melatonin regulates the viability and differentiation of rat midbrain neural stem cells, Cell Mol. Neurobiol. 28, 569–579.

Kukner, A., Colakoglu, N., Serin, D., Alagoz, G., Celebi, S., Kukner, A.S., 2006. Effects of intraperitoneal vitamin E, melatonin and aprotinin on leptin expression in the guinea pig eye during experimental uveitis. Acta Ophthalmol. Scand. 84, 54-61.

Kurz, S., Harrison, D.G., 1997. Insulin and the arginine paradox. J. Clin. Invest. 99, 369-370.

Leaden, P.J., Catala, A., 2007. Melatonin and N-acetyl serotonin inhibit selectively enzymatic and non-enzymatic lipid peroxidation of rat liver microsomes. Prostaglandins Leukot. Essent. Fatty Acids 77, 29-35.

Lee, P.Y., Podos, S.M., Serle, J.B., Camras, C.B., Severin, C.H., 1987. Intraocular pressure effects of multiple doses of drugs applied to glaucomatous monkey eyes. Arch. Ophthalmol. 105, 249-252.

Leon, J., Vives, F., Crespo, E., Camacho, E., Espinosa, A., Gallo, M.A., Escames, G., Acuña-Castroviejo, D., 1998. Modification of nitric oxide synthase activity and neuronal response in rat striatum by melatonin and kynurenine derivatives. J. Neuroendocrinol. 10, 297-302.

Leon, J., Acuña-Castroviejo, D., Sainz, R.M., Mayo, J.C., Tan, D.X., Reiter, R.J., 2004. Melatonin and mitochondrial function. Life Sci. 75, 765-790.

Leon, J., Acuña-Castroviejo, D., Escames, G., Tan, D.X., Reiter, R.J., 2005. Melatonin mitigates mitochondrial malfunction. J. Pineal Res. 38, 1-9.

Liu, B., Neufeld, A.H., 2000. Expression of nitric oxide synthase-2 (NOS-2) in reactive astrocytes of the human glaucomatous optic nerve head. Glia 30, 178-186.

Liu, C., Fukuhara, C., Wessel, J.H. III., Iuvone, P.M., Tosini, G., 2004. Localization of Aa-nat mRNA in the rat retina by fluorescence in situ hybridization and laser capture microdissection. Cell Tissue Res. 315, 197-201.

Liu, F., Yuan, H., Sugamori, K.S., Hamadanizadeh, A., Lee, F.J., Pang, S.F., Brown, G.M., Pristupa, Z.B., Niznik, H.B., 1995. Molecular and functional characterization of a partial cDNA encoding a novel chicken brain melatonin receptor. FEBS Lett. 374, 273-278.

Liu, J.H., Dacus, A.C., 1991. Endogenous hormonal changes and circadian elevation of intraocular pressure. Invest. Ophthalmol.Vis. Sci. 32, 496-500.

Long, D.J., Iskander, K., Gaikwad, A., Arin, M., Roop, D.R., Knox, R., Barrios, R., Jaiswal, A.K., 2002. Disruption of dihydronicotinamide riboside:quinone oxidoreductase 2 (NQO2) leads to myeloid hyperplasia of bone marrow and decreased sensitivity to menadione toxicity. J. Biol. Chem. 277, 46131-46139.

López, A., García, J.A., Escames, G., Venegas, C., Ortiz, F., López, L.C., Acuña-Castroviejo, D., 2009. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. J. Pineal Res. 46, 188-198.

Lundmark, P.O., Pandi-Perumal, S.R., Srinivasan, V., Cardinali, D.P., 2006. Role of melatonin in the eye and ocular dysfunctions. Vis. Neurosci. 23, 853-862.

Lundmark, P.O., Pandi-Perumal, S.R., Srinivasan, V., Cardinali, D.P., Rosenstein, R.E., 2007. Melatonin in the eye: implications for glaucoma. Exp. Eye Res. 84, 1021-1030.

Mailliet, F., Ferry, G., Vella, F., Berger, S., Coge, F., Chomarat, P., Mallet, C., Guenin, S.P., Guillaumet, G., Viaud-Massuard, M.C., Yous, S., Delagrange, P., Boutin, J.A., 2005. Characterization of the melatoninergic MT3 binding site on the NRH:quinone oxidoreductase 2 enzyme. Bioch. Pharmacol. 71, 74-88.

Manabe, S., Gu, Z., Lipton, S.A., 2005. Activation of matrix metalloproteinase-9 via neuronal nitric oxide synthase contributes to NMDA-induced retinal ganglion cell death. Invest. Ophthalmol. Vis. Sci. 46, 4747-4753.

Marchiafava, P.L., Longoni, B., 1999. Melatonin as an antioxidant in retinal photoreceptors. J. Pineal Res. 26, 184-189.

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 indoleamines in humans. Eur. J. Ophthalmol. 2, 67-72.

Martinez-Ruiz, A., Lamas, S., 2004. S-nitrosylation: a potential new paradigm in signal transduction. Cardiovasc. Res. 62, 43-52.

Melino, G., Bernassola, F., Knight, R.A., Corasaniti, M.T., Nistico, G., Finazzi-Agro, A., 1997. S-nitrosylation regulates apoptosis. Nature (London) 388, 432-433.

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

Mhatre, M.C., van Jaarsveld, A.S., Reiter, R.J., 1988. Melatonin in the lacrimal gland: first demonstration and experimental manipulation. Biochem. Biophys. Res. Commun. 153, 1186-1192.

Michel, T., Li, G.K., Busconi, L., 1993. Phosphorylation and subcellular translocation of endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. U.S.A. 90, 6252-6256.

Militante, J., Lombardini, J.B., 2004. Age-related retinal degeneration in animal models of aging: possible involvement of taurine deficiency and oxidative stress. Neurochem. Res. 29, 151-160.

Molinari, E.J., North, P.C., Dubocovich, M.L., 1996. 2-[125I]iodo-5-methoxycarbonylamino-N-acetyltryptamine: a selective radioligand for the characterization of melatonin ML2 binding sites. Eur. J. Pharmacol. 301, 159-168.

Moreno, M.C., Campanelli, J., Sande, P., Sanez, D.A., Keller Sarmiento, M.I., Rosenstein, R.E., 2004. Retinal oxidative stress induced by high intraocular pressure. Free Radic. Biol. Med. 37, 803-812.

Moreno, M.C., Sande, P., Marcos, H.A., deZavalía, N., Keller Sarmiento, M.I., Rosenstein, R.E., 2005. Effect of glaucoma on the retinal glutamate/glutamine cycle activity. FASEB J. 19, 1161-1162.

Namihira, M., Honma, S., Abe, H., Masubuchi, S., Ikeda, M., Honmaca, K., 2001. Circadian pattern, light responsiveness and localization of rPer1 and rPer2 gene expression in the rat retina. Neuroreport 12, 471-475.

Nathan, C., Xie, Q.W., 1994. Nitric oxide synthases: roles, tolls, and controls. Cell 78, 915-918.

Netland, P.A., Chaturvedi, N., Dreyer, E.B., 1993. Calcium channel blockers in the management of low-tension and open-angle glaucoma. Am. J. Ophthalmol. 115, 608-613.

Neufeld, A.H., Hernandez, M.R., Gonzalez, M., 1997. Nitric oxide synthase in the human glaucomatous optic nerve head. Arch. Ophthalmol. 115, 497-503.

Neufeld, A.H., 2004. Pharmacologic neuroprotection with an inhibitor of nitric oxide synthase for the treatment of glaucoma. Brain Res. Bull. 62, 455-459.

Niki, T., Hamada, T., Ohtomi, M., Sakamoto, K., Suzuki, S., Kako, K., Hosoya,Y., Horikawa, K., Ishida, N., 1998. The localization of the site of arylalkylamine N-acetyltransferase circadian expression in the photoreceptor cells of mammalian retina. Biochem. Biophys. Res. Commun. 248, 115-120.

Niles, L.P., Armstrong, K.J., Castro, L.M.R., Dao, C.V., Sharma, R., McMillan, C.R., Doering, L.C., Kirkham, D.L., 2004. Neural stem cells express melatonin receptors and neurotrophic factors: colocalization of the MT1 receptor with neuronal and glial markers. BMC Neurosci. 5, 41.

Nosjean, O., Ferro, M., Coge, F., Beauverger, P., Henlin, J.M., Lefoulon, F., Fauchere, J.L., Delagrange, P., Canet, E., Boutin, J.A., 2000. Identification of the melatonin-binding site MT3 as the quinone reductase 2. J. Biol. Chem. 275, 31311-31317.

Osborne, N.N., 1994. Serotonin and melatonin in the iris/ciliary processes and their involvement in intraocular pressure. Acta Neurobiol. Exp.(Wars.) 54 Suppl, 57-64.

Osborne, N.N., Nash, M.S., Wood, J.P., 1998. Melatonin counteracts ischemia-induced apoptosis in human retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 39, 2374-2383.

Osborne, N.N., Chidlow, G., Nash, M.S., Wood, J.P., 1999. The potential of neuroprotection in glaucoma treatment. Curr. Opin. Ophthalmol. 10, 82-92.

Oxenkrug, G., 2005. Antioxidant effects of N-acetylserotonin: possible mechanisms and clinical implications. Ann. N. Y. Acad. Sci. 1053, 334-347.

Pandi-Perumal, S.R., Srinivasan, V., Maestroni, G.J., Cardinali, D.P., Poeggeler, B., Hardeland, R., 2006. Melatonin: Nature's most versatile biological signal? FEBS J. 273, 2813-2838.

Pang, I.H., Johnson, E.C., Jia, L., Cepurna, W.O., Shepard, A.R., Hellberg, M.R., Clark, A.F., Morrison, J.C., 2005. Evaluation of inducible nitric oxide synthase in glaucomatous optic neuropathy and pressure-induced optic nerve damage. Invest. Ophthalmol. Vis. Sci. 46, 1313-1321.

Pang, S.F., Yu, H.S., Suen, H.C., Brown, G.M., 1980. Melatonin in the retina of rats: a diurnal rhythm. J. Endocrinol. 87, 89-93.

Papp, A., Nemeth, I., Karg, E., Papp, E., 1999. Glutathione status in retinopathy of prematurity. Free Radic. Biol. Med. 27, 738-743.

Perianayagam, M.C., Oxenkrug, G.F., Jaber, B.L., 2005. Immune-modulating effects of melatonin, N-acetylserotonin, and N-acetyldopamine. Ann. N. Y. Acad. Sci. 1053, 386-393.

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, 671-689.

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.

Planck, S.R., Huang, X.N., Robertson, J.E., Rosenbaum, J.T., 1994. Cytokine mRNA levels in rat ocular tissues after systemic endotoxin treatment. Invest. Ophthalmol. Vis. Sci. 35, 924-930.

Poliandri, A.H., Esquifino, A.I., Cano, P., Jimenez, V., Lafuente, A., Cardinali, D.P., Duvilanski, B.H., 2006. In vivo protective effect of melatonin on cadmium-induced changes in redox balance and gene expression in rat hypothalamus and anterior pituitary. J. Pineal Res. 41, 238-246.

Pozdeyev, N., Taylor, C., Haque, R., Chaurasia, S.S., Visser, A., Thazyeen, A., Du, Y., Fu, H., Weller, J., Klein, D.C., Iuvone, P.M., 2006. Photic regulation of arylalkylamine N-acetyltransferase binding to 14-3-3 proteins in retinal photoreceptor cells. J. Neurosci. 26, 9153-9161.

Pozo, D., Reiter, R.J., Calvo, J.R., Guerrero, J.M., 1997. Inhibition of cerebellar nitric oxide synthase and cyclic GMP production by melatonin via complex formation with calmodulin. J. Cell Biochem. 65, 430-442.

Quigley, H.A., McKinnon, S.J., Zack, D.J., Pease, M.E., Kerrigan-Baumrind, L.A., Kerrigan, D.F., Mitchell, R.S., 2000. Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest. Ophthalmol. Vis. Sci. 41, 3460–3466.

Rada, J.A., Wiechmann, A.F., 2006. Melatonin receptors in chick ocular tissues: implications for a role of melatonin in ocular growth regulation. Invest. Ophthalmol. Vis. Sci. 47, 25-33.

Reiter, R.J., Tan, D.X., Poeggeler, B., Menendez-Pelaez, A., Chen, L.D., Saarela, S., 1994. Melatonin as a free radical scavenger: implications for aging and age-related diseases. Ann. N.Y. Acad. Sci. 719, 1-12.

Reiter, R.J., Guerrero, J.M., Escames, G., Pappolla, M.A., Acuña-Castroviejo, D., 1997. Prophylactic actions of melatonin in oxidative neurotoxicity. Ann. N. Y. Acad. Sci. 825, 70-78.

Reiter, R.J., Garcia, J.J., Pie, J., 1998. Oxidative toxicity in models of neurodegeneration: responses to melatonin. Restor. Neurol. Neurosci. 12, 135-142.

Reiter, R.J., Cabrera, J., Sainz, R.M., Mayo, J.C., Manchester, L.C., Tan, D.X., 1999. Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism. Ann. N. Y. Acad. Sci. 890, 471-485.

Reiter, R.J., Tan, D.X., Osuna, C., Gitto, E., 2000. Actions of melatonin in the reduction of oxidative stress. A review. J. Biomed. Sci. 7, 444-458.

Reiter, R.J., Tan, D.X., Manchester, L.C., Simopoulos, A.P., Maldonado, M.D., Flores, L.J., Terron, M.P., 2007. Melatonin in edible plants (phytomelatonin): Identification, concentrations, bioavailability and proposed functions. World Rev. Nutr. Diet. 97, 211-230.

Reppert, S.M., Godson, C., Mahle, C.D., Weaver, D.R., Slaugenhaupt, S.A., Gusella, J.F., 1995. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc. Natl. Acad. Sci. U. S. A. 92, 8734-8738.

Rodriguez, I.R., Mazuruk, K., Schoen, T.J., Chader, G.J., 1994. Structural analysis of the human hydroxyindole-O-methyltransferase gene. Presence of two distinct promoters. J. Biol. Chem. 269, 31969-31977.

Rosenstein, R., Belforte, N., de Zavalía, N., Keller Samiento, M.I., Moreno, M.C., 2009. Melatonin prevents functional and histological alterations in an experimental model of glaucoma in rats. Invest. Ophthalmol. Vis. Sci. 50, E-Abstract 2765.

Rufiange, M., Dumont, M., Lachapelle, P., 2002. Correlating retinal function with melatonin secretion in subjects with an early or late circadian phase. Invest. Ophthalmol. Vis. Sci. 43, 2491-2499.

Sáenz, D.A., Turjanski, A.G., Sacca, G.B., Marti, M., Doctorovich, F., Sarmiento, M.I., Estrin, D.A., Rosenstein, R.E., 2002. Physiological concentrations of melatonin inhibit the nitridergic pathway in the Syrian hamster retina. J. Pineal Res. 33, 31-36.

Sáenz, D.A., Goldin, A.P., Minces, L., Chianelli, M., Sarmiento, M.I., Rosenstein, R.E., 2004. Effect of melatonin on the retinal glutamate/glutamine cycle in the golden hamster retina. FASEB J. 18, 1912-1913.

Samples, J.R., Krause, G., Lewy, A.J., 1988. Effect of melatonin on intraocular pressure. Curr. Eye Res. 7, 649-653.

Sande, P.H., Fernandez, D.C., Aldana Marcos, H.J., Chianelli, M.S., Aisemberg, J., Silberman, D.M., Saenz, D.A., Rosenstein, R.E., 2008. Therapeutic effect of melatonin in experimental uveitis. Am. J. Pathol. 173, 1702-1713.

Sasaki, M., Ozawa, Y., Kurihara, T., Noda, K., Imamura, Y., Kobayashi, S., Ishida, S., Tsubota, K., 2009. Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. Invest. Ophthalmol. Vis. Sci. 50, 1433-1439.

Savaskan, E., Wirz-Justice, A., Olivieri, G., Pache, M., Krauchi, K., Brydon, L., Jockers, R., Muller-Spahn, F., Meyer, P., 2002a. Distribution of melatonin MT1 receptor immunoreactivity in human retina. J. Histochem. Cytochem. 50, 519-526.

Savaskan, E., Wirz-Justice, A., Olivieri, G., Pache, M., Krauchi, K., Brydon, L., Jockers, R., Muller-Spahn, F., Meyer, P., 2002b. Distribution of melatonin MT1 receptor immunoreactivity in human retina. J. Histochem. Cytochem. 50, 519-526.

Savaskan, E., Jockers, R., Ayoub, M., Angeloni, D., Fraschini, F., Flammer, J., Eckert, A., Muller-Spahn, F., Meyer, P., 2007. The MT2 melatonin receptor subtype is present in human retina and decreases in Alzheimer's disease. Curr. Alzheimer Res. 4, 47-51.

Scher, J., Wankiewicz, E., Brown, G.M., Fujieda, H., 2002. MT(1) melatonin receptor in the human retina: expression and localization. Invest. Ophthalmol. Vis. Sci. 43, 889-897.

Scher, J., Wankiewicz, E., Brown, G.M., Fujieda, H., 2003. AII amacrine cells express the MT1 melatonin receptor in human and macaque retina. Exp. Eye Res. 77, 375-382.

Serle, J.B., Podos, S.M., Kitazawa, Y., Wang, R.F., 1998. A comparative study of latanoprost (Xalatan) and isopropyl unoprostone (Rescula) in normal and glaucomatous monkey eyes. Jpn. J. Ophthalmol. 42, 95-100.

Serle, J.B., Wang, R.F., Peterson, W.M., Plourde, R., Yerxa, B.R., 2004. Effect of 5-MCA-NAT, a putative melatonin MT3 receptor agonist, on intraocular pressure in glaucomatous monkey eyes. J. Glaucoma 13, 385-388.

Siu, A.W., Reiter, R.J., To, C.H., 1999. Pineal indoleamines and vitamin E reduce nitric oxide-induced lipid peroxidation in rat retinal homogenates. J. Pineal Res. 27, 122-128.

Siu, A.W., Ortiz, G.G., Benitez-King, G., To, C.H., Reiter, R.J., 2004. Effects of melatonin on the nitric oxide treated retina. Br. J. Ophthalmol. 88, 1078-1081.

Siu, A.W., Maldonado, M., Sanchez-Hidalgo, M., Tan, D.X., Reiter, R.J., 2006. Protective effects of melatonin in experimental free radical-related ocular diseases. J. Pineal Res. 40, 101-109.

Smith, S.D., Gregory, D.S., 1989. A circadian rhythm of aqueous flow underlies the circadian rhythm of IOP in NZW rabbits. Invest. Ophthalmol. Vis. Sci. 30, 775-778.

Srinivasan, V., Pandi-Perumal, S.R., Maestroni, G.J., Esquifino, A.I., Hardeland, R., Cardinali, D.P., 2005. Role of melatonin in neurodegenerative diseases. Neurotox. Res. 7, 293-318.

Srinivasan, V., Pandi-Perumal, S.R., Cardinali, D.P., Poeggeler, B., Hardeland, R., 2006. Melatonin in Alzheimer's disease and other neurodegenerative disorders. Behav. Brain Funct. 2, 15.

Stevens, B.R., Kakuda, D.K., Yu, K., Waters, M., Vo, C.B., Raizada, M.K., 1996. Induced nitric oxide synthesis is dependent on induced alternatively spliced CAT-2 encoding L-arginine transport in brain astrocytes. J. Biol. Chem. 271, 24017-24022.

Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., Reiter, R.J., 2007a. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J. Pineal Res. 42, 28-42.

Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., Tamura, H., Reiter, R.J., 2007b. Melatonin as a naturally occurring co-substrate of quinone reductase-2, the putative MT3 melatonin membrane receptor: hypothesis and significance. J. Pineal Res. 43, 317-320.

Tang, G.Y., Ip, A.K., Siu, A.W., 2007. Pinoline and N-acetylserotonin reduce glutamate-induced lipid peroxidation in retinal homogenates. Neurosci. Lett. 412, 191-194.

Tang, Q., Hu, Y., Cao, Y., 2006. Neuroprotective effect of melatonin on retinal ganglion cells in rats. J. Huazhong Univ. Sci. Technolog. Med. Sci. 26, 235-7, 253.

Tate, D.J.Jr., Miceli, M.V., Newsome, D.A., 1995. Phagocytosis and H2O2 induce catalase and metallothionein gene expression in human retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 36, 1271-1279.

Thompson, C.L., Selby, C.P., Partch, C.L., Plante, D.T., Thresher, R.J., Araujo, F., Sancar, A., 2004. Further evidence for the role of cryptochromes in retinohypothalamic photoreception/phototransduction. Brain Res. Mol. Brain Res. 122, 158-166.

Tosini, G., Menaker, M., 1996. Circadian rhythms in cultured mammalian retina. Science 272, 419-421.

Tosini, G., Menaker, M., 1998. The clock in the mouse retina: melatonin synthesis and photoreceptor degeneration. Brain Res. 789, 221-228.

Tosini, G., 2000. Melatonin circadian rhythm in the retina of mammals. Chronobiol. Int. 17, 599-612.

Tosini, G., Chaurasia, S.S., Michael, I.P., 2006. Regulation of arylalkylamine N-acetyltransferase (AANAT) in the retina. Chronobiol. Int. 23, 381-391.

Tosini, G., Pozdeyev, N., Sakamoto, K., Iuvone, P.M., 2008. The circadian clock system in the mammalian retina. Bioessays 30, 624-633.

Turjanski, A.G., Estrin, D.A., Rosenstein, R.E., McCormick, J.E., Martin, S.R., Pastore, A., Biekofsky, R.R., Martorana,V., 2004. NMR and molecular dynamics studies of the interaction of melatonin with calmodulin. Protein Sci. 13, 2925-2938.

van Reyk, D.M., Gillies, M.C., Davies, M.J., 2003. The retina: oxidative stress and diabetes. Redox Rep. 8, 187-192.

Vorwerk, C.K., Lipton, S.A., Zurakowski, D., Hyman, B.T., Sabel, B.A., Dreyer, E.B., 1996. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. Invest. Ophthalmol. Vis. Sci. 37, 1618-1624.

Vorwerk, C.K., Hyman, B.T., Miller, J.W., Husain, D., Zurakowski, D., Huang, P.L., Fishman, M.C., Dreyer, E.B., 1997. The role of neuronal and endothelial nitric oxide synthase in retinal excitotoxicity. Invest. Ophthalmol. Vis. Sci. 38, 2038-2044.

Weber, A.J., Harman, C.D., Viswanathan, S., 2008. Effects of optic nerve injury, glaucoma, and neuroprotection on the survival, structure, and function of ganglion cells in the mammalian retina. J. Physiol. 586, 4393-4400.

Wiechmann, A.F., 1986. Melatonin: parallels in pineal gland and retina. Exp. Eye Res. 42, 507-527.

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

Wiechmann, A.F., Rada, J.A., 2003. Melatonin receptor expression in the cornea and sclera. Exp. Eye Res. 77, 219-225.

Wiechmann, A.F., Udin, S.B., Summers Rada, J.A., 2004. Localization of Mel1b melatonin receptor-like immunoreactivity in ocular tissues of Xenopus laevis. Exp. Eye Res. 79, 585-594.

Wiechmann, A.F., Summers, J.A., 2008. Circadian rhythms in the eye: the physiological significance of melatonin receptors in ocular tissues. Prog. Retin. Eye Res. 27, 137-160.

Witkovsky, P., Veisenberger, E., Lesauter, J., Yan, L., Johnson, M., Zhang, D.Q., McMahon, D., Silver, R., 2003. Cellular location and circadian rhythm of expression of the biological clock gene Period 1 in the mouse retina. J. Neurosci. 23, 7670-7676.

Yilmaz, T., Celebi, S., Kukner, A.S., 2002. The protective effects of melatonin, vitamin E and octreotide on retinal edema during ischemia-reperfusion in the guinea pig retina. Eur. J. Ophthalmol. 12, 443-449.

Yu, H.S., Yee, R.W., Howes, K.A., Reiter, R.J., 1990. Diurnal rhythms of immunoreactive melatonin in the aqueous humor and serum of male pigmented rabbits. Neurosci. Lett. 116, 309-314.

Yulek, F., Or, M., Ozogul, C., Isik, A.C., Ari, N., Stefek, M., Bauer, V., Karasu, C., 2007. Effects of stobadine and vitamin E in diabetes-induced retinal abnormalities: involvement of oxidative stress. Arch. Med. Res. 38, 503-511.

Zhou, L., Li, Y., Yue, B.Y., 1999. Oxidative stress affects cytoskeletal structure and cell-matrix interactions in cells from an ocular tissue: the trabecular meshwork. J. Cell Physiol. 180, 182-189.

Zisapel, N., 2001. Melatonin-dopamine interactions: from basic neurochemistry to a clinical setting. Cell Mol. Neurobiol. 21, 605-616.

LEGENDS

Figure 1

Schematic representation of melatonin receptor distribution in ocular tissues.

Figure 2

Mechanisms involved in glaucomatous RGC apoptosis. Chronic ocular hypertension increases NO production, oxidative stress, glutamate synaptic levels, a vascular dysregulation leading to ischemia, as well as a decrease in neurotrophin levels. All these mechanisms can be targeted by melatonin in an opposite way to that induced by glaucoma.

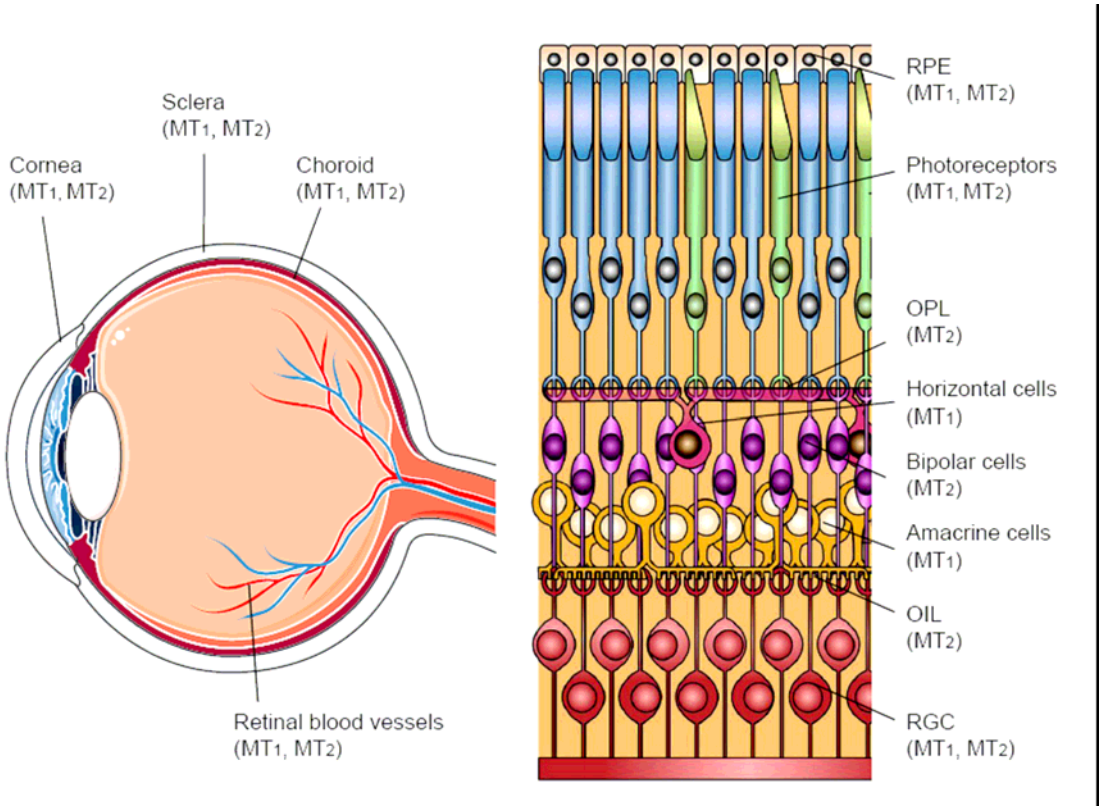
Sclera
(MT1, MT2)
Cornea
(MT1, MT2)
Choroid
(MT1, MT2)
Retinal blood vessels
(MT1, MT2)
RPE
(MT1, MT2)
Photoreceptors
(MT1, MT2)
OPL
(MT2)
Horizontal cells
(MT1)
Bipolar cells
(MT2)
Amacrine cells
(MT1)
OIL
(MT2)
RGC
(MT1, MT2)

Figure 1

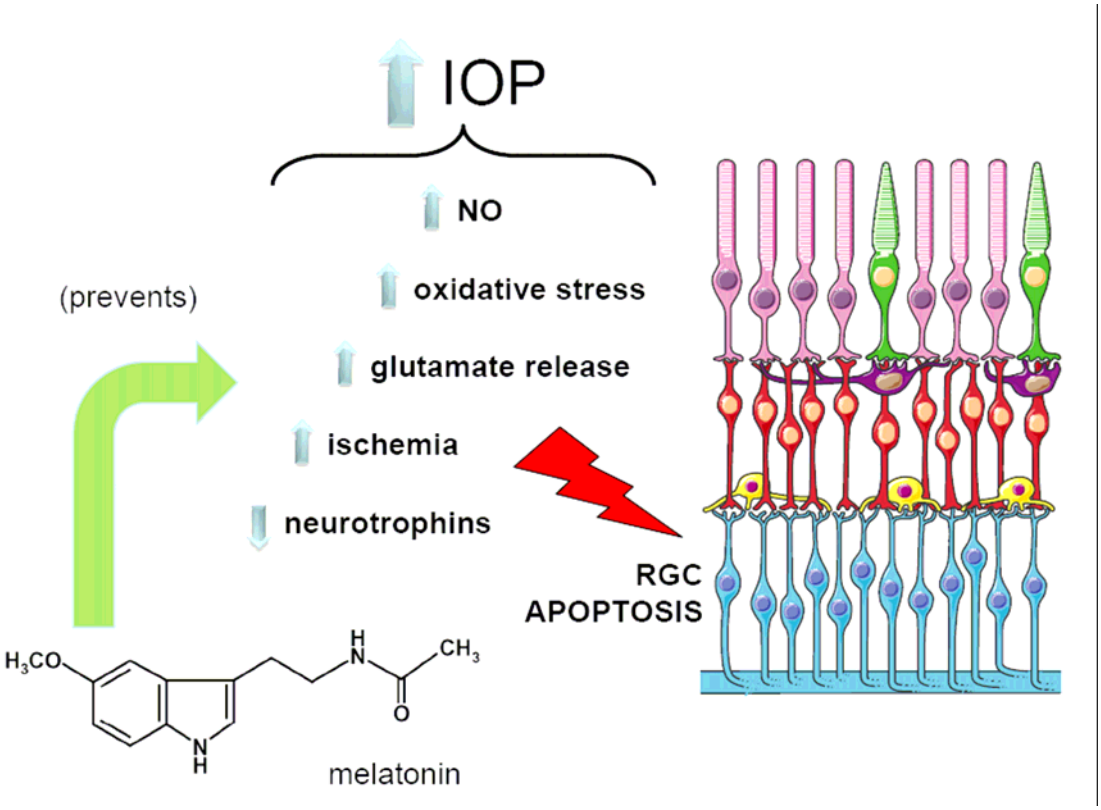
IOP
NO
oxidative stress
glutamate release
ischemia
neurotrophins
(prevents)
RGC
APOPTOSIS
H3CO
CH3
melatonin

Figure 2