Investigative Ophthalmology & Visual Science, Vol. 33, No. 6, May 1992

Melatonin Increases Photoreceptor Susceptibility to Light-Induced Damage

Allan F. Wiechmann and W. Keith O'Steen

Melatonin is an indolamine hormone synthesized in the retina and pineal gland. It is thought to act as a paracrine neurohormone in the mammalian retina. Pinealectomy has been shown to protect photoreceptors from light-induced damage, and melatonin treatment has been reported to increase the degree of photoreceptor damage in albino rats. To determine how melatonin influences photoreceptor survival, the effect of melatonin administration on light-induced retinal damage was studied. Melatonin was administered to albino rats by intraperitoneal injections at various times before or after light exposure. The rats were exposed to high-intensity illumination (1600 lux) for 24 hr to induce photodamage, then returned to cyclic lighting for 12 days. After this, they were killed, and their eyes were removed and examined histologically. Measurements of the outer nuclear layer (ONL) thickness were taken at 12 different loci around the circumference of the retinal sections. The animals that received daily melatonin injections (100 μg) in the late afternoon (3 hr before lights off) for 1–3 days before photodamage showed an approximate 30% greater reduction compared with sham control animals in ONL thickness in the superior quadrant, the area most susceptible to light damage. Melatonin injections given after the photodamage did not affect ONL thickness. Although retinal susceptibility to light damage varied with time of day, the degree to which melatonin increased the degree of damage appeared unaffected by the time of day. These results suggest that melatonin may be involved in some aspects of photoreceptor sensitivity to light damage. Invest Ophthalmol Vis Sci 33:1894–1902, 1992

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized cyclically, with peak levels during the dark period, in the pineal gland and retina of most species studied.[1] Melatonin produced by the pineal gland acts as an endocrine hormone involved in the regulation of seasonal reproduction and pigmentation; melatonin of retinal origin is thought to act as a paracrine neurohormone with local effects in the retina.[2] Studies from several laboratories suggest that retinal melatonin may be involved in photoreceptor outer segment disc shedding and phagocytosis,[3–5] retinomotor movements,[6] retinal pigment epithelium cell membrane potential,[7] and modulation of dopamine release.[8] This substance also may influence intraocular pressure,[9] photoreceptor susceptibility to light damage,[10,11] and the sensitivity of retinal neurons to light.[12–14] The mechanism of melatonin action in the retina is not known, but it may act through antagonism of the dopamine system. The site of action of melatonin in the retina is thought to be the inner plexiform layer.[15,16]

Serotonin (5-hydroxytryptamine) is converted to N-acetyl-5-hydroxytryptamine by the enzyme serotonin N-acetyltransferase (NAT).[17] Retinal NAT activity is highest during the dark.[18,19] N-Acetyl-5-hydroxytryptamine is converted to melatonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT).[20] Melatonin-like immunofluorescence has been detected in the outer nuclear layer (ONL) and inner nuclear layer of the retina,[21] and HIOMT-like immunoreactivity has been localized to the retinal photoreceptors and a subpopulation of cone bipolar cells in several species, including rats and humans.[22–25] These studies suggest that photoreceptors and some bipolar cells may be the sites of melatonin synthesis in the retina.

The observations by others[10,11] that melatonin increases the degree of light-induced photoreceptor damage supports earlier studies[26] that pinealectomized rats show less photoreceptor damage than do sham control animals. Because one function of melatonin may be to increase the sensitivity of the retina to light,[12–14,27] as part of a dark-adaptation mechanism, an undesirable consequence of this may be an increased sensitivity to the damaging effects of light. We

From the Departments of Neurobiology & Anatomy and Ophthalmology and Program in Neuroscience, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina.

Supported by research grants EY08006 (AFW) and AG07767 (WKO) from the National Institutes of Health (Bethesda, Maryland).

Submitted for publication: June 19, 1991; accepted November 14, 1991.

Reprint requests: Dr. Allan F. Wiechmann, Department of Neurobiology & Anatomy, Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1010.

conducted this series of experiments to confirm the earlier reports of an effect of melatonin on light-induced retinal damage in albino rats and to characterize the temporal conditions under which melatonin exerts its effects.

Materials and Methods

Animal Maintenance and Light Exposures

Adult male Sprague-Dawley rats (8 weeks old; Zivic-Miller, Allison Park, PA) were maintained on a cyclic photoperiod (14-hr light and 10-hr darkness; lights on at 5 AM, and lights off at 7 PM) at 21°C for 2 weeks in an animal room adjacent to the laboratory. The illuminance intensity during the light period was 80 lux. Water and Purina rat chow (St. Louis, Missouri) were provided ad libitum. After drug treatments, groups of animals were placed in polycarbonate cages with wire tops and minimal bedding and exposed to 24 hr of continuous light. Except where indicated for one diurnal experiment, the animals were placed into the continuous lighting at 4 PM (3 hr before lights off). The high-intensity illumination (HII) to which the animals were exposed was 1600 lux of fluorescent light as measured with a Tektronix (Morrisville, NC) J16 digital photometer with the illuminance probe located at animal eye level and directed toward the light source. The exposure chambers were 54-cm^2 wooden boxes provided with continuous air exchange. Typically, four animals (representing two different experimental groups) were placed in one chamber. Temperature during HII exposure was maintained at 30 ± 1.5°C. After HII exposure, the rats were returned to cyclic lighting for 12 days to allow phagocytic removal of dead and damaged cells from the retina.[28] In most experiments, a group of non-HII-exposed animals remained in the cyclic lighting room for the duration of the experiment, and their eyes were removed and processed with the experimental tissues. The animals were killed with ether vapors, and the eyes were enucleated and placed in fixative, dehydrated, and embedded in paraffin.

The use of animals in this investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Melatonin Treatments

Melatonin (Sigma, St. Louis, MO) was administered to the animals by intraperitoneal injections as in earlier studies.[10,11] Melatonin was dissolved in a small volume of absolute ethanol, then diluted to a concentration of 1 mg/ml in saline. The final ethanol concentration injected into the animals was less than 2%; therefore, less than 2 μl of ethanol per injection was given. Control animals received sham injections containing the identical amount of ethanol and saline given to the melatonin-treated groups.

In the first experiment, 100 μg of melatonin was given at 4 PM for 3 days before exposure to HII and for 12 days after HII. Therefore, these animals received daily injections (at 4 PM) for 15 days continuously. The animals were exposed to 24 hr (ie, from 4 PM to 4 PM) of HII immediately after the third injection. This dosage and injection schedule was similar to that used in earlier studies.[10,11]

The relationship between melatonin effectiveness and timing of the melatonin treatment relative to HII exposure should provide some insight into the mechanism by which melatonin exerts its effects on the retina. Therefore, melatonin (100 μg) injections were given at 4 PM for either 3 days before (pre-HII group) or 12 days after (post-HII group) HII exposure to determine the period during which melatonin is most effective in influencing photoreceptor cell death. The pre-HII group received sham injections during the 12-day HII recovery period. Similarly, the post-HII group received sham injections during the 3 days before HII exposure. Thus, both groups received either a melatonin or sham injection each of the 15 days of the experiment to control for stress caused by the injection.

After determining that melatonin treatment before HII exposure was more effective than after HII exposure, the animals were given melatonin injections (100 μg) for either 3 days (once a day at 4 PM) or for 1 day only (immediately before light dosing) before HII exposure to determine if a single melatonin injection would elicit a response. A third group of animals was given sham injections for 3 days before HII exposure.

In another experiment, groups of rats were injected with 100 μg of melatonin or received sham injections at 7 AM (2 hr after lights on) or 5 PM (2 hr before lights off) and then placed immediately into HII for 24 hr to determine if melatonin effectiveness varies with time of day. After HII, the animals were returned to cyclic lighting (80 lux) for 12 days.

The dose of melatonin given to all rats in these experiments was 100 μg because that was the amount used in previous studies.[10,11] To determine if other doses of melatonin were effective in increasing light-induced retinal damage, groups of rats were injected once with 10, 100, or 1000 μg of melatonin immediately before HII exposure. A fourth group of animals was given a sham injection before HII exposure, and another uninjected group remained in the cyclic lighting room (unexposed control).

Morphometric Analysis of Retinal Damage

The eyes were enucleated from animals killed by ether vapors 12 days after HII exposure to allow for

phagocytic removal of dead and damaged cells from the retina.[28] Before removal, the superior surface of the eyes was marked with an indelible pen for future orientation during sectioning and measurement. The eyes were fixed in Perfix histologic fixative (Fisher, Pittsburgh, PA) for 4 hr, dehydrated in an ethanol and xylene series, and embedded in paraffin. Tissue blocks were sectioned at 7 μm on the anterior–posterior axis, and sagittal sections of the retina (including the optic nerve) were stained with Harris' hematoxylin and eosin for morphologic analysis. Measurements of total retinal thickness and ONL thickness were made on each retina at 12 different loci around the circumference of each section with an ocular micrometer as described previously.[28] This method of linear thickness measurement has been verified by others as an efficient and accurate method of quantitative assessment of photoreceptor layer integrity.[29] Statistical significance was determined by comparing data from the various groups of animals in each experiment by paired or unpaired student t-test, in which a single determination was based on an average of the two eyes from a single animal.

Melatonin Radioimmunoassay of Serum and Retina

To determine the absolute levels and duration of the drug delivered to the rat retina after a 100-μg injection, a melatonin radioimmunoassay (RIA) was done on rat retina and serum at various times after injection. Melatonin (100 μg) was administered to groups of four rats by intraperitoneal injections early during the light period. The rats were anesthetized with ether vapors and decapitated at selected times after drug administration (0, 0.5, 1, 2, 4, 8, 12, and 24 hr). Trunk blood was collected, and the retinas were removed. The serum and isolated retinas were stored at −80°C until assay.

The tissues were assayed using a melatonin RIA described previously.[30] Both retinas from each animal were pooled and sonicated together in 500 μl of phosphate-buffered saline. Aliquots of serum (50 or 500 μl) or whole retina homogenate were transferred to 16 × 100-mm glass tubes, then 2 ml of chloroform was added, and the samples were vortex mixed for 30 sec. The aqueous phase was aspirated, and 1 ml of the organic phase (containing the melatonin) was transferred to 12 × 75-mm glass tubes and evaporated to dryness with nitrogen gas. The samples were resuspended in 0.5 ml of 0.05 mol/l sodium phosphate-buffered saline containing thimerosal 0.01% and gelatin 0.1% (Sigma) pH 7.5 (RIA buffer) and vortex mixed for 10 sec. To each sample, we added 0.1 ml of rabbit antimelatonin antibody (CIDtech, Mississauga, Canada) and 0.05 ml of O-methyl-^{3}H-melatonin (approximately 15,000 disintegrations per minute per sample; 78.4 Ci/mmol; Amersham, Arlington Heights, IL). The mixture was incubated for 20 hr at 4°C, then 0.65 ml of saturated ammonium sulfate (pH 7) was added to the samples, which were vortex mixed twice. The samples were incubated at 4°C for 1 hr, then centrifuged at 5000 × g for 30 min at 4°C. The supernatant was aspirated, and the pellets were resuspended in 0.5 ml of water and vortex mixed. The samples were placed in scintillation vials, 10 ml of Ecolume scintillation cocktail (ICN, Lisle, IL) was added, and the radioactivity was measured. Melatonin values were obtained from a melatonin standard curve.

Results

Albino rats exposed to 24 hr of HII developed irreversible photoreceptor damage, as measured by a reduction in ONL thickness (Fig. 1). Because the cell bodies of the retinal photoreceptors are located in the ONL, the thickness of this layer is a direct reflection of photoreceptor cell death.[31] Retinal damage was limited to the outer retina (ONL and outer and inner segments), and no obvious reduction in the thickness of the inner retinal cell layers was observed, although a quantitative analysis of other layers was not undertaken. Unexposed control animals (maintained on cyclic lighting) consistently showed modest ONL damage in the superior hemisphere of the retina, presumably incurred before delivery from the supplier. A greater sensitivity to light damage in the superior hemisphere of the retina, as contrasted with the inferior half, has been described.[32] Throughout the course of this study, we observed some variability in ONL thickness among unexposed rats. Although there were statistically significant differences from one experiment to another between unexposed control groups at some retinal loci, most of the retinal loci among such control animals in the different experiments did not show statistically significant differences in ONL thickness. There appeared to be more inherent ONL thickness variability in the superior hemisphere than in the inferior hemisphere.

Albino rats receiving 100-μg melatonin injections for 3 days before and 12 days after HII exposure showed a higher degree of ONL damage than the sham-injected control group (Fig. 2, $P < 0.005$). The superior hemisphere had a greater sensitivity to melatonin than did the inferior hemisphere ($P < 0.01$). The differences between sham and melatonin-injected groups were statistically significant in both the superior hemisphere (retinal loci, 1–6; $P < 0.05$) and the inferior hemisphere (retinal loci, 7–12; $P < 0.02$) when analyzed by a paired Student's t-test.

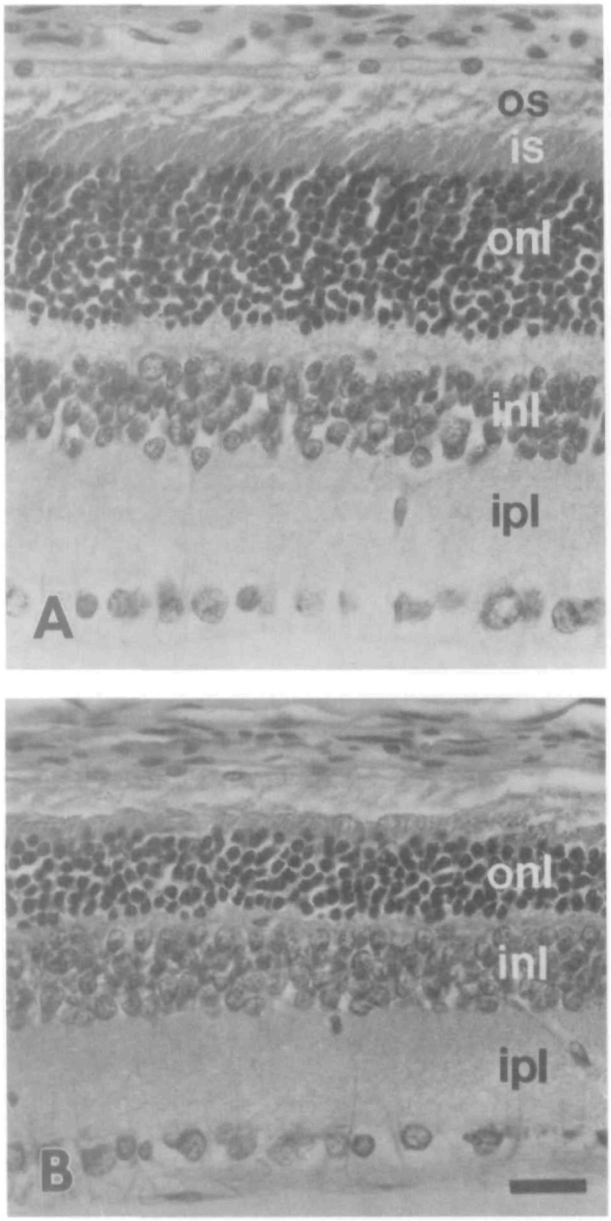

Fig. 1. Light micrographs of albino rat retinas exposed to cyclic and high-intensity illumination. (**A**) Superior retina of albino rat exposed to normal cyclic lighting. (**B**) Superior hemisphere of albino rat retina 12 days after exposure to 24-hr continuous high-intensity illumination (HII; 1600 lux, 30°C). Note the thinning of the outer nuclear layer. Photoreceptor outer segments (os), photoreceptor inner segments (is), outer nuclear layer (onl), inner nuclear layer (inl), inner plexiform layer (ipl). Magnification bar represents 20 μm.

To determine the temporal pattern of melatonin exposure that would lead to a decrease in photoreceptor survival, rats were injected with 100 μg of melatonin for 3 days before HII or for 12 days after HII. Animals receiving melatonin injections before HII (pre-HII group) showed a greater reduction in ONL thickness than those that received melatonin injections after HII (post-HII group, $P < 0.001$, Fig. 3). These results indicated that melatonin exerts its effects at some time before or during the light exposure, not during the phagocytic recovery period after light exposure.

To determine the minimal requirement of melatonin exposure needed to affect photoreceptor damage, rats were injected for 3 days before HII (the rats were placed in HII immediately after the third injection) or injected once immediately before HII exposure. Retinas of rats injected for 3 days ($P < 0.05$) or 1 day ($P < 0.01$) before HII showed greater reduction of ONL thickness in the superior hemisphere than control-injected animals (Fig. 4). Rats receiving only one injection immediately before HII exposure, however, showed a greater degree of damage in the superior hemisphere than did the rats receiving melatonin treatment for 3 days ($P < 0.025$). The inferior hemisphere was unaffected by melatonin treatment. These results demonstrate that a single injection of melatonin immediately before HII exposure is sufficient to exert a deleterious effect on photoreceptor survival in

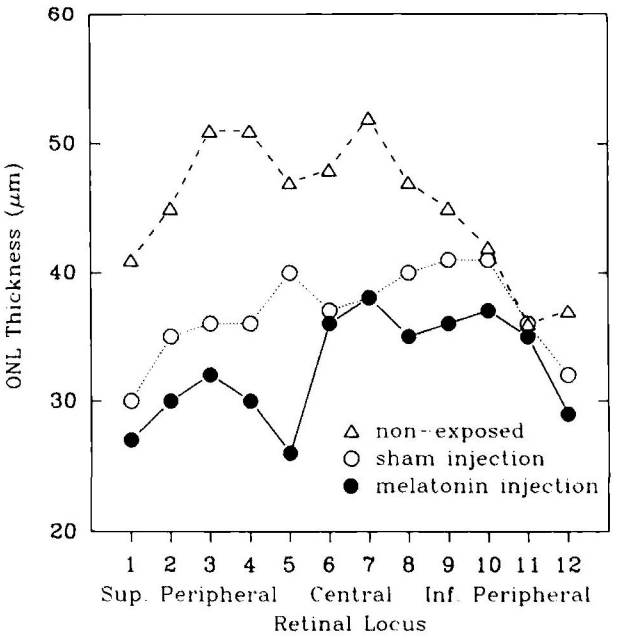

Fig. 2. Outer nuclear layer (ONL) thickness of rats exposed to high-intensity illumination (HII) for 24 hr. Measurements were made at 12 different loci of the superior (sup.) and inferior (inf.) peripheral and central retina. Melatonin injections given for 3 days prior to and for 2 weeks after HII cause a greater reduction in photoreceptor nuclei (ONL) in the superior retina, compared to the exposed sham controls ($P < 0.005$). The sham and melatonin-treated groups each consisted of eight animals. One nonexposed animal was maintained in cyclic lighting for the duration of the experiment. At retinal locus 5, the ONL thickness of the melatonin-treated group was 26 ± 3 μm SEM, compared to 40 ± 1 μm SEM for the sham group.

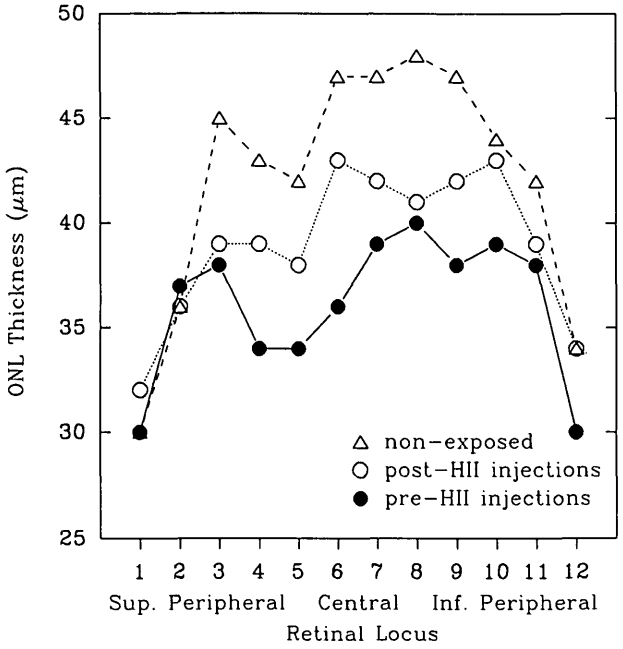

Fig. 3. Rats receiving melatonin injections for 3 days prior to HII (pre-HII group, n = 8) show a greater degree of ONL damage ($P < 0.001$), compared to animals receiving melatonin injections for 12 days following exposure to HII (post-HII group, n = 8). Most of the damage appears in the superior hemisphere of the pre-HII group. At retinal loci 4, 5, and 6, the ONL thickness was 34 ± 5 μm SEM, 34 ± 5 μm, and 36 ± 7 μm, respectively, for the pre-HII group, and was 39 ± 5 μm, 38 ± 2 μm, and 43 ± 6 μm, respectively, for the post-HII group. Four nonexposed animals were maintained in cyclic lighting for the duration of the experiment.

the superior hemisphere and that this dose is more effective than three daily melatonin treatments before HII.

A single melatonin injection was given to groups of rats in the late afternoon (5 PM, 2 hr before lights off, Fig. 5) or early morning (7 AM, 2 hr after lights on, Fig. 6). Then the rats were exposed immediately to HII for 24 hr. The untreated (sham) early morning (7 AM) group appeared to be more sensitive to HII than the untreated late afternoon (5 PM) group ($P < 0.001$). Melatonin treatment reduced ONL thickness when given at either 7 AM ($P < 0.001$) or 5 PM ($P < 0.01$). The percent differences between the sham and melatonin-injected groups were similar at both times (8–10%). However, since the 7 AM sham control group showed a higher degree of damage than the 5 PM control group, it follows that the 7 AM melatonin-treated group showed a higher degree of ONL damage than the 5 PM melatonin-treated group ($P < 0.001$). Therefore, melatonin treatment early in the light period results in greater reduction of ONL thickness than treatments given late in the light period.

Single injections of 10, 100, or 1000 μg of melatonin were given to groups of rats to determine the effectiveness of melatonin doses other than the 100-μg dose used in other studies.[10,11] Although all three doses of melatonin resulted in a higher degree of ONL damage than the sham-injected group, the 10-μg dose produced the most damage (Fig. 7).

Intraperitoneal injection of 100 μg of melatonin resulted in a peak level of melatonin of about 4400 pg/ml serum at 15 min (Fig. 8), as measured by RIA. Serum melatonin levels declined quickly and reached the daytime baseline level within 2 hr. Similarly, levels of immunoreactive melatonin in the retina peaked at 15 min, with a peak value of about 63 pg/retina (Fig. 9). Retinal melatonin levels also reached baseline within 2 hr.

Discussion

This series of experiments demonstrated that melatonin treatment increases the susceptibility of retinal photoreceptors to light-induced damage. Rats exposed to HII after intraperitoneal injection of melatonin consistently had significantly thinner ONL thicknesses than did animals receiving control vehicle injections. These results confirm earlier reports that melatonin increases light-induced photoreceptor damage in albino rats[10,11] and further demonstrate that a brief early exposure of melatonin during HII is sufficient to exert a deleterious effect on photoreceptor

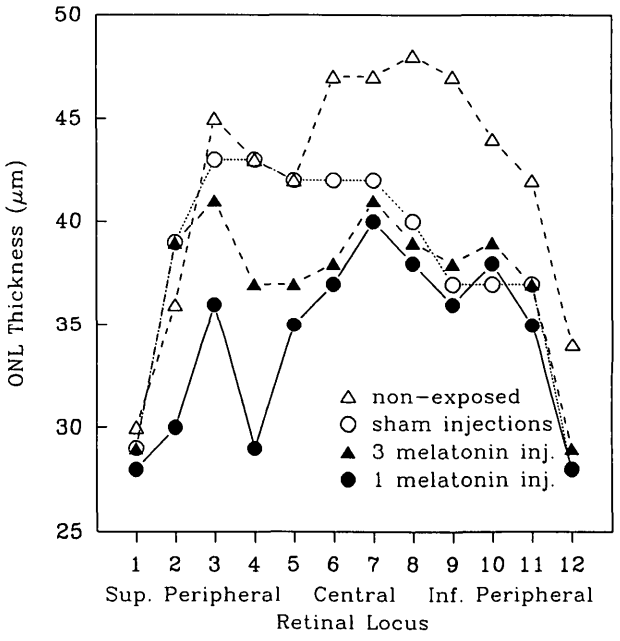

Fig. 4. Rats receiving a single melatonin injection immediately prior to HII show a greater degree of ONL damage (thinning) in the superior region, compared to animals receiving melatonin injections for 3 days prior to HII exposure ($P < 0.025$). At retinal locus 4, the ONL thickness of the sham group was 43 ± 2 μm SEM, compared to 29 ± 5 μm and 37 ± 3 μm for the single injection and 3-day injection groups, respectively. The melatonin-treated groups each consisted of six animals, and the sham-injected and nonexposed groups each consisted of four animals.

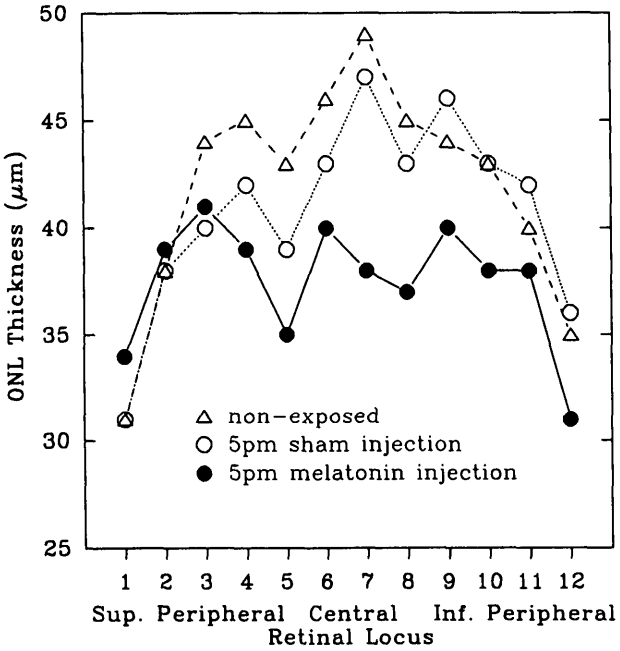

Fig. 5. Effect of melatonin treatment given in late afternoon on ocular light damage. The rats were entrained to a 14L:10D light/dark cycle (lights on: 5 AM, lights off: 7 PM), then given a 100-μg melatonin or sham injection at 5 PM (2 hr before lights off), and placed immediately into HII for 24 hr. The sham-injected group shows very little damage, compared to nonexposed controls. The melatonin-treated group shows a greater degree of damage then the sham-injected group ($P < 0.01$). The melatonin-treated group consisted of six animals, the sham group had four animals, and the nonexposed group had five animals.

survival. It has been shown previously that melatonin has no effect on ONL thickness in non-HII exposed retinas.[11]

Melatonin is synthesized by both the pineal gland and retina cyclically, with peak levels during the dark.[2] The immunocytochemical localization of HIOMT (the melatonin-synthesizing enzyme) in pinealocytes[22,33] supported the belief that these cells are responsible for melatonin synthesis in the pineal gland. Also, the presence of HIOMT-like immunoreactivity in the photoreceptors and in some cone bipolar cells in the retina of several species, including rats and humans, suggested that these cells may be the sites of retinal melatonin synthesis.[22-25]

Others[26] have shown previously that pinealectomized albino rats have less photoreceptor damage than do intact sham-pinealectomized rats when both groups are exposed to HII, suggesting that the major hormonal product of the pineal gland, melatonin, may play a role in light-induced ocular damage. Our results support these findings. Similarly, removal of the pituitary gland results in a protection of the retinal photoreceptors in rats exposed to HII compared with that observed in the retinas of intact rats.[34] When the effects of prolactin administration to hypophysectomized rats were examined, it was found that prolactin was capable of reversing the protection of the retinal photoreceptors from photic destruction offered by pituitary ablation.[35] The observation (that melatonin stimulates the release of prolactin in rats[36,37]) suggests that the effect of melatonin on ocular light damage may be mediated by prolactin. Autoradiographic studies have suggested the presence of prolactin receptors on the photoreceptor inner segments of the rat retina.[38] The site of action of melatonin in the retina is thought to be the inner plexiform layer.[15,16] Bromocriptine, a dopamine D_2 agonist, is reported to protect photoreceptors from light-induced damage.[10] Because melatonin inhibits dopamine release in the retina,[8] the effect of melatonin on light damage may be mediated through an inhibition of a protective effect of dopamine. The presence of putative melatonin receptors in the inner plexiform layer (the location of dopaminergic amacrine cell processes) and dopamine receptors in the photoreceptor layers[39-42] would support this possibility. Also, because dopamine inhibits prolactin release from the pituitary,[43] the protective effect of bromocriptine may be mediated by a decrease in prolactin levels in the blood, resulting in a protection of the retina from light-induced damage.

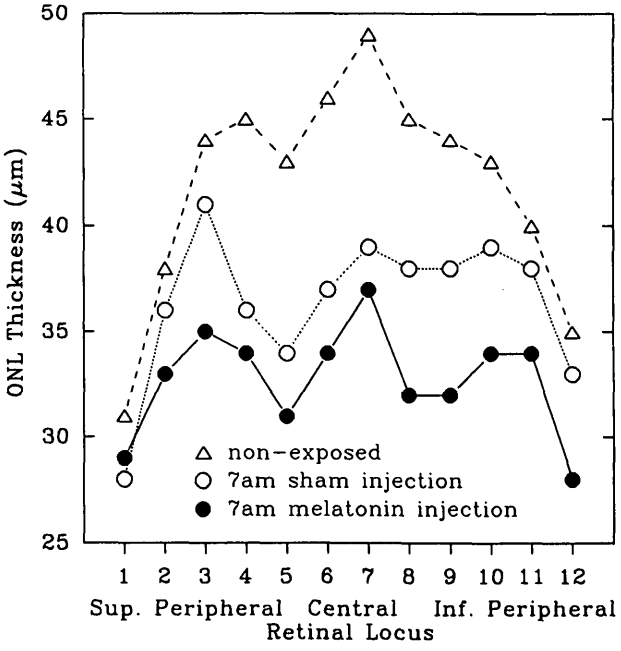

Fig. 6. Effect of melatonin treatment given in early morning on ocular light damage. Rats were treated as described in Figure 5, except that the injections were administered at 7 AM (2 hr after lights on). Both the sham-injected and melatonin-injected groups show a greater degree of damage than the nonexposed controls, with the melatonin-treated group showing more damage than the sham-injected group ($P < 0.001$). Both the sham and melatonin-treated groups show more ONL damage ($P < 0.001$) than the corresponding groups injected at 5 PM (Fig. 5). The number of animals in each group was the same as in Figure 5.

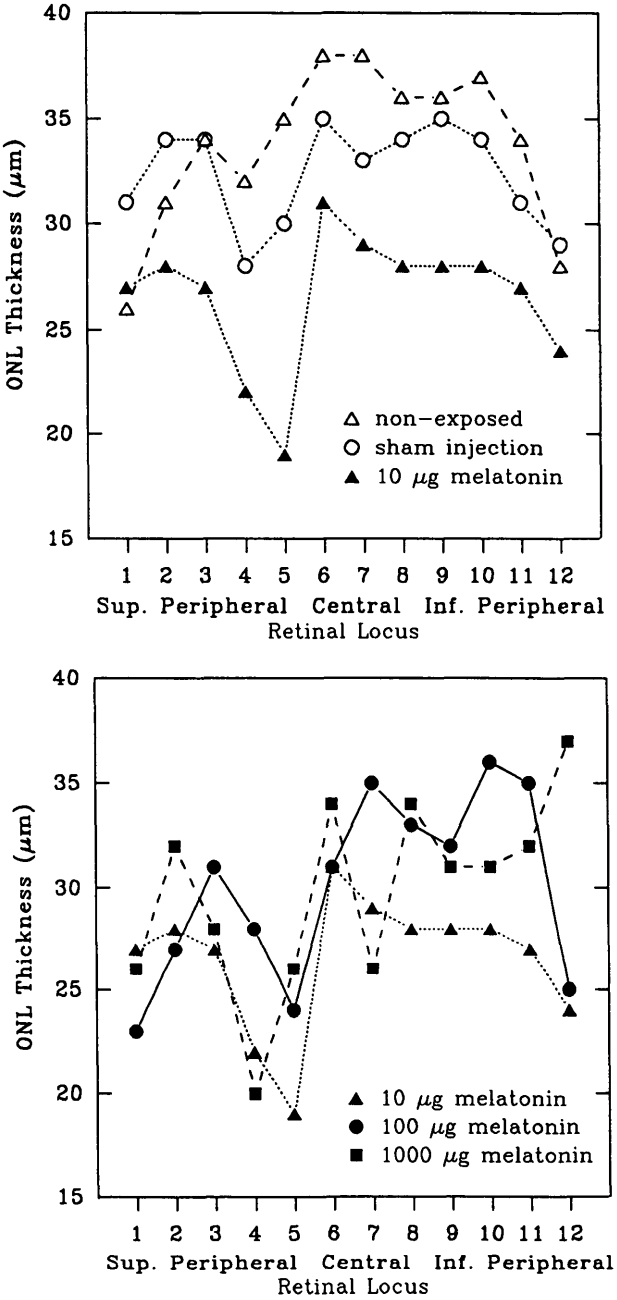

Fig. 7. Dose–response of HII-induced photoreceptor damage to various concentrations of melatonin. A single injection of melatonin or vehicle (sham) was given just prior to exposure to HII. *Upper graph:* A 10-μg melatonin injection results in considerable thinning of the ONL, especially in the superior region. *Lower graph:* In the same experiment as in the upper graph, 100 μg and 1000 μg melatonin also result in thinning of the ONL in the superior region of the retina. The dosage of 10 μg melatonin was most effective in inducing photoreceptor damage, and the 100-μg dosage was least effective in inducing damage. Each group consisted of four animals.

Whether the effects of melatonin on light-induced photoreceptor damage are the result of its direct action on the retina or are mediated by stimulation of prolactin release or inhibition of dopamine release is a subject for additional study.

The observation that a single injection of melatonin immediately before HII increases photoreceptor damage and that melatonin treatment given after HII has little or no effect on ONL thickness indicates that melatonin exerts its effects during the period of HII exposure, rather than during the recovery period after HII exposure. Furthermore, we found a single injection of melatonin had a greater effect on photoreceptor damage than three successive daily injections; prior exposure to melatonin may desensitize the target tissue (ie, retina or pituitary) to later melatonin

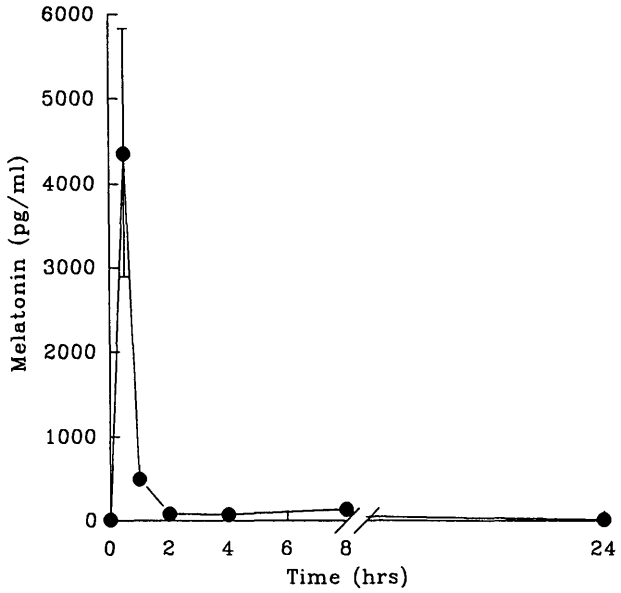

Fig. 8. Serum concentrations of melatonin in male rats at 0 to 24 hr following a single intraperitoneal injection of 100 μg melatonin, as measured by radioimmunoassay. Each data point represents the mean values from three animals ± SEM.

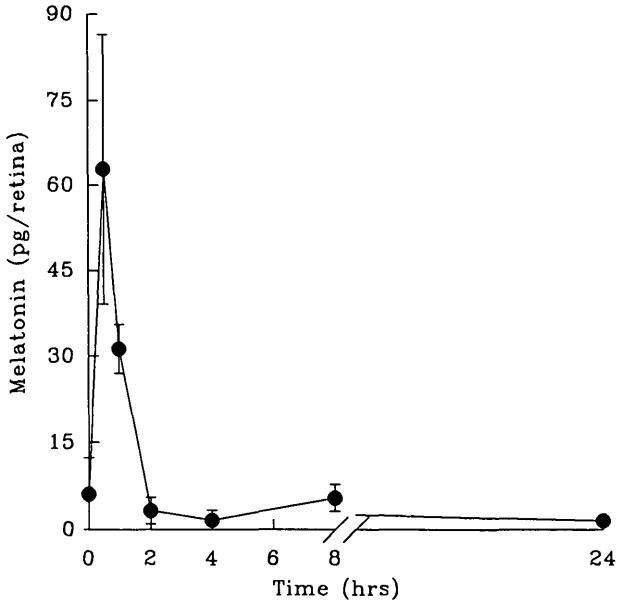

Fig. 9. Melatonin concentrations in rat retina at 0 to 24 hr following a single intraperitoneal injection of 100 μg melatonin. Each data point represents the mean values from three animals ± SEM.

treatment. The phenomenon of insensitivity to melatonin after prior melatonin treatment has been reported for the rodent reproductive system.[44,45] However, rats given a 100-mg subcutaneous melatonin implant for 5 days before HII exposure had reduced ONL thickness in the inferior hemisphere of the retina (unpublished observations).

A diurnal variation in the susceptibility of photoreceptors to light-induced cell death has been reported previously,[46] and it was confirmed by our study. Photoreceptor cells are more susceptible to damage in the early light period and less susceptible late in the light period. Although early morning melatonin treatment results in more photoreceptor damage than late afternoon treatment, the reduction in ONL thickness relative to the appropriate control treatments was similar at both times. This suggests that the diurnal variation in sensitivity to melatonin in the retina may merely reflect the diurnal sensitivity of the retina to other factors responsible for initiating cell death. This contrasts with the diurnal rhythm in sensitivity of the hamster reproductive system to melatonin, in which late afternoon melatonin treatment is effective in inhibiting luteinizing hormone and follicle-stimulating hormone release, and early morning treatment has no effect.[47]

Melatonin RIAs were done on rat retina and serum at various times after melatonin injection to determine the absolute levels of melatonin in the retina as a function of time and dose. Injection of 100 μg of melatonin resulted in approximately 4400 pg melatonin/ml serum and 60 pg/retina. The reported nighttime peaks of endogenous melatonin are approximately 120 pg/ml serum[48] and 150 pg/retina[49] in the rat. The melatonin levels in the retina resulting from intraperitoneal injection of 100 μg are therefore within the physiologic range expected for a direct action of melatonin on the retina. Because injection of 10 μg of melatonin was apparently more effective than 100 μg in increasing photoreceptor susceptibility to light-induced damage, the resulting intraocular levels may be substantially lower, perhaps below the physiologic range for a direct action of melatonin on the retina. A tenfold decrease in serum melatonin concentration, however, would apparently be in a physiologic range for an indirect action of melatonin on the retina, perhaps mediated by prolactin. These questions will be the subject of future study.

Previous studies suggested that melatonin was produced by the photoreceptors and a subpopulation of cone bipolar cells in both rats and humans.[22-24] The rat retina does not have a foveal region, and the HIOMT immunoreactive cone bipolar cells are distributed evenly throughout the albino rat retina.[25] In human retina, photoreceptors, especially cones, are much more numerous in the foveal region than in the peripheral region. Also, the HIOMT-immunoreactive cone bipolar cells are more numerous in the foveal region.[23] This high density of melatonin-synthesizing cells in the foveal region of the human retina may result in higher local concentrations of melatonin in this region compared with the peripheral retina. Therefore, because melatonin increases the susceptibility of photoreceptors to light-induced degeneration, the foveal (or macular) region may be more susceptible to damage if melatonin acts directly on the retina. Under conditions of artificial lighting, humans may be subjected to inappropriately high levels of ocular or circulating melatonin while exposed to environmental light because light intensities similar to those used in our studies on albino rats do not significantly suppress the nocturnal rise in serum melatonin levels in humans.[50] In disease states, there may be an increased risk of selective degeneration of the macular region of the retina caused by the damaging effects of melatonin during exposure to light. It was hypothesized that circadian organization arose from a requirement for certain chemical activities to avoid potentially harmful radiant energy of the sun.[51] The presence of a "dark" chemical signal during light exposure therefore may disrupt a temporal organization required for normal cell function.

Key words: retina, light damage, melatonin, albino rat, photoreceptor

Acknowledgments

The authors thank Lisa Beatty and Jody Bowman for excellent technical assistance.

References

1. Pang SF and Allen AE: Extra-pineal melatonin in the retina: Its regulation and physiological function. Pineal Research Reviews 4:55, 1986.
2. Wiechmann AF: Melatonin: Parallels in pineal gland and retina. Exp Eye Res 42:507, 1986.
3. Besharse JC and Dunis DA: Methoxyindoles and photoreceptor metabolism: Activation of rod shedding. Science 219:1341, 1983.
4. White MP and Fisher LJ: Effects of exogenous melatonin on circadian disc shedding in the albino rat retina. Vision Res 29:167, 1989.
5. Ogino N, Matsumura M, Shirakawa H, and Tsukahara I: Phagocytic activity of cultured retinal pigment epithelial cells from chick embryo: Inhibition by melatonin and cyclic AMP, and its reversal by taurine and cyclic GMP. Ophthalmic Res 15:72, 1983.
6. Pierce ME and Besharse JC: Circadian regulation of retinomotor movements. J Gen Physiol 86:671, 1985.
7. Nao-i N, Nilsson SEG, Gallemore RP, and Steinberg RH: Effects of melatonin on the chick retinal pigment epithelium: Membrane potentials and light-evoked responses. Exp Eye Res 49:573, 1989.
8. Dubocovich ML: Melatonin is a potent modulator of dopamine release in the retina. Nature 306:782, 1983.
9. Chiou GCY, Aimoto T, and Chiou LY: Melatonergic involvement in diurnal changes of intraocular pressure in rabbit eyes. Ophthalmic Res 17:373, 1985.

10. Bubenik GA and Purtill RA: The role of melatonin and dopamine in retinal physiology. Can J Physiol Pharmacol 58:1457, 1980.
11. Leino M, Aho I-M, Kari E, Gynther J, and Markkanen S: Effects of melatonin and 6-methoxy-tetrahydro-β-carboline in light induced retinal damage: A computerized morphometric method. Life Sci 35:1997, 1984.
12. Wiechmann AF, Yang X-L, Wu SM, and Hollyfield JG: Melatonin enhances horizontal cell sensitivity in salamander retina. Brain Res 453:377, 1988.
13. Reuss S and Kiefer W: Melatonin administered systemically alters the properties of visual cortex cells in cat: Further evidence for a role in visual information processing. Vision Res 29:1089, 1989.
14. Semm P and Vollrath L: Alterations in the spontaneous activity of cells in the guinea pig pineal gland and visual system produced by pineal indoles. J Neural Transm 53:265, 1982.
15. Laitinen JT and Saavedra JM: The chick retinal melatonin receptor revisited: Localization and modulation of agonist binding with guanine nucleotides. Brain Res 528:349, 1990.
16. Wiechmann AF and Wirsig-Wiechmann CR: Localization and quantification of high-affinity melatonin binding sites in *Rana pipiens* retina. J Pineal Res 10:174, 1991.
17. Klein DC and Weller JL: Indole metabolism in the pineal gland: A circadian rhythm in N-acetyltransferase. Science 169:1093, 1970.
18. Hamm HE and Menaker M: Retinal rhythms in chicks: Circadian variation in melatonin and serotonin N-acetyltransferase activity. Proc Natl Acad Sci USA 77:4998, 1980.
19. Iuvone PM and Besharse JC: 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, 1983.
20. Axelrod J and Weissbach H: Enzymatic O-methylation of N-acetylserotonin to melatonin. Science 131:1312, 1960.
21. Bubenik GA, Purtill RA, Brown GM, and Grota LJ: Melatonin in the retina and the harderian gland: Ontogeny, diurnal variations and melatonin treatment. Exp Eye Res 27:323, 1978.
22. Wiechmann AF, Bok D, and Horwitz J: Localization of hydroxyindole-O-methyltransferase in the mammalian pineal gland and retina. Invest Ophthalmol Vis Sci 26:253, 1985.
23. Wiechmann AF and Hollyfield JG: Localization of hydroxyindole-O-methyltransferase-like immunoreactivity in photoreceptors and cone bipolar cells in the human retina: A light and electron microscope study. J Comp Neurol 258:253, 1987.
24. Wiechmann AF and Hollyfield JG: HIOMT-like immunoreactivity in the vertebrate retina: A species comparison. Exp Eye Res 49:1079, 1989.
25. Wiechmann AF and O'Steen WK: Hydroxyindole-O-methyltransferase in rat retinal bipolar cells: Persistence following photoreceptor destruction. Brain Res 506:14, 1990.
26. Rudeen PK and O'Steen WK: The effects of the pineal gland on light-induced retinal photoreceptor damage. Exp Eye Res 28:37, 1979.
27. Souêtre E, De Galeani B, Gastaud P, Salvati E, and Darcourt G: 5-Methoxypsoralen increases the sensitivity of the retina to light in humans. Eur J Clin Pharmacol 36:59, 1989.
28. O'Steen WK and Donnelly LE: Chronologic analysis of variations in retinal damage in to strains of rats after short-term illumination. Invest Ophthalmol Vis Sci 22:252, 1982.
29. Michon JJ, Zi-liang L, Shioura N, Anderson RJ, and Tso MOM: A comparative study of methods of photoreceptor morphometry. Invest Ophthalmol Vis Sci 32:280, 1991.
30. Wiechmann AF, Kyritsis AP, Fletcher RT, and Chader GJ: Cyclic AMP and butyrate modulate melatonin synthesis in Y79 human retinoblastoma cells. J Neurochem 55:208, 1990.
31. Noell WK, Walker VS, Kang BS, and Berman S: Retinal damage by light in rats. Invest Ophthalmol 5:450, 1966.
32. Rapp LM and Williams TP: A parametric study of retinal light damage in albino and pigmented rats. *In* The Effects of Constant Light on Visual Processes, Williams TP and Baker BB, editors. New York, Plenum, 1980, pp. 135–159.
33. Kuwano R, Iwanaga T, Nakajima T, Masuda T, and Takahashi Y: Immunocytochemical demonstration of hydroxyindole-O-methyltransferase (HIOMT), neuron-specific enolase (NSE) and S-100 protein in the bovine pineal gland. Brain Res 274:171, 1983.
34. Olafson RP and O'Steen WK: Hormonal influences on photoreceptor damage: The pituitary gland and ovaries. Invest Ophthalmol 15:869, 1976.
35. O'Steen WK and Kraeer SL: Effects of hypophysectomy, pituitary gland homogenates and transplants, and prolactin on photoreceptor destruction. Invest Ophthalmol 16:940, 1977.
36. Kamberi IA, Mical RS, and Porter JC: Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88:1288, 1971.
37. Lu K-H and Meites J: Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology 93:152, 1973.
38. O'Steen WK and Sundberg DK: Patterns of radioactivity in the eyes of rats after injection of iodinated prolactin. Ophthalmic Res 14:54, 1982.
39. Brann MR and Young WS: Dopamine receptors are located on rods in bovine retina. Neurosci Lett 69:221, 1986.
40. Stormann TM, Gdula DC, Weiner DM, and Brann MR: Molecular cloning and expression of a dopamine D2 receptor from human retina. Mol Pharmacol 37:1, 1990.
41. Denis P, Elena P-P, Nordmann J-P, Saraux H, and Lapalus P: Autoradiographic localization of D1 and D2 dopamine binding sites in the human retina. Neurosci Lett 116:81, 1990.
42. Schorderet M and Nowak JZ: Retinal dopamine D1 and D2 receptors: Characterization by binding or pharmacological studies and physiological functions. Cell Mol Neurobiol 10:303, 1990.
43. Fluckiger E: Effects of bromocriptine on the hypothalamo-pituitary axis. Acta Endocrinol (Copenh) 88:111, 1978.
44. Reiter RJ: The pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1:109, 1980.
45. Cardinali DP: Melatonin: A mammalian pineal hormone. Endocr Rev 2:327, 1981.
46. Duncan TE and O'Steen WK: The diurnal susceptibility of rat retinal photoreceptors to light-induced damage. Exp Eye Res 41:497, 1985.
47. Tamarkin L, Westrom WK, Hamill AI, and Goldman BD: Effect of melatonin on the reproductive systems of male and female hamsters: A diurnal rhythm in sensitivity to melatonin. Endocrinology 99:1534, 1976.
48. Wilkinson M, Arendt J, Bradtke J, and deZiegler D: Determination of a dark-induced increase of pineal N-acetyltransferase activity and simultaneous radioimmunoassay of melatonin in pineal, serum and pituitary tissue of the male rat. J Endocrinol 72:243, 1977.
49. Pang SF, Yu HS, Suen HC, and Brown GM: Melatonin in the retina of rats: A diurnal rhythm. J Endocrinol 87:89, 1980.
50. Boyce P and Kennaway DJ: Effects of light on melatonin production. Biol Psychiatry 22:473, 1987.
51. Pittendrigh CS: Biological clocks: The functions, ancient and modern, of circadian oscillations. *In* Science in the Sixties: Proceedings of the 1965 Cloudcraft Symposium. Air Force Office of Scientific Research, 1965, pp. 96–109.