Agonists at the Serotonin Receptor (5-HT₁A) Protect the Retina from Severe Photo-Oxidative Stress

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PURPOSE. 5-HT₁A agonists are neuroprotective in CNS injury models. The authors evaluated the efficacy of 5-HT₁A agonists to protect the retina from severe blue light-induced photo-oxidative damage.

METHODS. Albino rats were dosed (subcutaneously) with AL-8309A, 8-OH DPAT, or buspironne once or three times before 6-hour exposure to blue light. Electroretinograms (ERGs) were measured to assess retinal function, and retinal damage was evaluated by light microscopy. Topical ocular dosing with 1.75% AL-8309B was also evaluated. Rats were dosed with WAY-100635, a 5-HT₁A antagonist, to determine whether protection required activation of the 5-HT₁A receptor.

RESULTS. ERG response amplitudes were significantly (P < 0.05) depressed more than 66% in vehicle-dosed rats after light exposure. ERGs were significantly higher in rats treated with AL-8309A (0.1–30 mg/kg), 8-OH DPAT (0.1–1 mg/kg), buspironne (5–20 mg/kg) or topical ocular with 1.75% AL-8309B. Retinas from AL-8309A and 8-OH DPAT–treated rats were devoid of histologic lesions. Significant protection was measured in rats dosed once 0, 24, or 48 hours before light exposure. Protection provided by dosing with AL-8309B or 8-OH DPAT was inhibited in rats predosed with WAY-100635.

CONCLUSIONS. 5-HT₁A agonists provided potent and complete functional and structural protection. Protection was inhibited by treatment with WAY-100635, confirming the requirement for activating the 5-HT₁A receptor in initiating this survival pathway. Single-dose experiments with AL-8309A suggest that the mechanism of protection is rapidly activated and protection persists for 48 hours. AL-8309B (1.75%) was effective after topical ocular dosing. AL-8309B is under evaluation in the clinic and may be useful in treating age-related macular degeneration. (Invest Ophthalmol Vis Sci. 2011;52:2118–2126) DOI:10.1167/iovs.10-6504

Acute exposure of rodents to photo-oxidative stress results in retinal degeneration exhibiting features of atrophic age-related macular degeneration (AMD), including photoreceptor and RPE degeneration, choriocapillaris atrophy, and retinal remodeling. Short-wavelength light, under certain exposure conditions, has the greatest phototoxicity potential of the visible-light spectrum. Although the chromophores mediating blue light damage have not been conclusively determined, injury may result from the generation of reactive oxygen species (ROS) by mitochondrial cytochromes, inhibition of cytochrome oxidase and adenosine triphosphate depletion, leading to calcium accumulation and apoptosis. Damage may be mediated through rhodopsin given that blue light has been shown to increase the photon-catch capability of photoreceptors because of the photoreversal of bleaching. A2E is another potential source of blue light–mediated ROS that can lead to apoptosis of RPE cells. Blue light–damaged rat retinas also exhibit complement activation. Serotonin (5-hydroxytryptamine or 5-HT) and its multiple receptors are known to regulate various physiological functions. 5-HT₁A receptor agonists play an integral role in the control of sleep, feeding, and anxiety. Two 5-HT₁A agonists, buspironne and tandospironne, are prescribed for the treatment of anxiety disorders. 5-HT₁A receptor agonists are also neuroprotective. In vitro, 5-HT₁A agonists provide protection against a variety of insults, including serum deprivation, staurosporine, anoxia, and ethanol-induced apoptosis, excito-toxicity, and hydrogen peroxide–induced toxicity, and in a model of compressive spinal cord injury. Similarly, 5-HT₁A agonists are neuroprotective in animal models of CNS ischemia, acute subdermal hematoa, traumatic brain injury, excitotoxicity, MPTP-toxicity models of Parkinson’s, sciatic nerve crush, delayed progression of motor neuron degeneration in pmm mice, and reduced lipid peroxidation in a rat epilepsy model.

The present study aimed to determine whether selected 5-HT₁A agonists could protect the retina from the severe photo-oxidative injury induced by exposure to blue light. If 5-HT₁A agonists were neuroprotective, we wanted to determine whether this effect was mediated by 5-HT₁A receptors, how rapidly 5-HT₁A-mediated neuroprotective mechanisms are activated, the duration of this activity, and whether drugs of this class can be delivered to the retina by topical ocular application. Some of this work has been previously described in abstract form (Collier RJ, et al. IOVS 2009;50:ARVO E-Abstract 675).

SUBJECTS, MATERIALS, AND METHODS

Subjects and Dosing

Male Sprague-Dawley rats (weight range, 300–450 g) were randomly assigned to control (not light-exposed), vehicle-dosed, and drug-dosed
groups. Control rats were housed under broad-band fluorescent (Sylvania Cool White, 45 ft-c) cyclic light (12 hours light:12 hours dark). Rats in this group were not exposed to blue light and were not dosed with vehicle or drug. Food and water were available ad libitum to all experimental groups. All experimental procedures, as well as animal care and handling, adhered to the guidelines outlined in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All experimental procedures were also reviewed and approved by our Institutional Animal Care and Use Committee.

Three 5-HT₁₄ agonists—AL-8309A, 8-OH DPAT, and buspirone—were evaluated to determine the dose-dependent protection provided by this class of pharmacologic agents. 8-OH DPAT is in a different structural class from buspirone and AL-8309A. To determine the significance of 5-HT₁₄ receptor activation, WAY-100655, a selective 5-HT₁₄ receptor antagonist, was evaluated. AL-8309A (provided by Dainippon Sumitomo, Osaka, Japan) and AL-8309B (Alcon Research Ltd., Fort Worth, TX) have the same structure and are two different salt forms of the same molecule. All other drugs were from Sigma-Aldrich (St. Louis, MO).

In most experiments, drugs were dissolved in sterile 0.9% sodium chloride and administered by subcutaneous injection according to the following schedule: once daily starting 2 days before light exposure, once immediately before light exposure, and once daily 1 and 2 days after light exposure. In some experiments, drugs were administered only once, at the indicated time before light exposure. The viability of the topical ocular route of administration was also evaluated. Rats were dosed topical ocular starting 21 days before light exposure and for 2 days after light exposure. Dosing occurred once or twice a day using 1.75% AL-8309B. AL-8309B (HCl salt) was used rather than the AL-8309A (citrate salt) because of its better solubility at physiological pH.

Induction of Photochemical Lesions

Rats were exposed to light as previously described. Rats were dark adapted for 24 hours before the induction of photochemical lesions. Photo-oxidative induced lesions were generated by exposure to blue light (3.1 mW/cm², Philips fluorescent lamps [F40/BB]) for 6 hours. Rats were single housed in clear polycarbonate cages with minimal bedding to prevent burrowing. Movement of rats within the cage was unrestricted during this light exposure. Animals were allowed to recover for 5 days in darkness before electrodiagnostic evaluation of retinal function. Animals were then returned to their home cages for an additional 3-week recovery period in cyclic light (12 hours light:12 hours dark) before retinal function was reassessed and ocular tissues were harvested for morphologic evaluation.

Electrodiagnostic Evaluation

Decline in retinal function was assessed by examining the electoretinogram (ERG). ERGs were recorded from dark-adapted anesthetized rats (ketamine HCl 75 mg/kg, xylazine 6 mg/kg, administered intraperitoneally). Animal preparation before ERG recording was performed using a photographic safe-light. Corneas were anesthetized (Alcaine; Alcon Laboratories, Fort Worth, TX), and pupils were dilated (Neo-Synephrine [Sanoft, New York, NY] and Mydriacyl [Alcon]). Anesthetized rats were positioned on a circulating hot water heating pad (35°C) to maintain normal body temperature while recording ERGs. Flash ERGs were recorded from a platinum-iridium wire-loop electrode positioned on the cornea. A tungsten eyelid speculum was used as a reference electrode, and a ground electrode was attached to the ipsilateral pinna. Hydroxypropylmethylcellulose (2%) was applied to the cornea to minimize corneal dehydration. The head of the rat was held in a standardized position, and the eye from which recordings were to be made was placed in a Ganzfeld bowl illuminator. Rats were given an additional 30-minute dark-adaptation period before ERGs were elicited. Electrical signals were amplified (P15 AC amplifier; Grass, Quincy, MA), bandpass filtered from 0.3 Hz to 10 kHz, and digitized (DAS 1600; Keithley Instruments, Cleveland, OH). Light-evoked electrical responses were elicited by a 10-μs duration flash generated by a xenon flash tube (PS22, Grass). Light intensity was controlled by insertion of neutral density filters. ERGs for some experiments were performed using an ERG system (Espion; Diagnosys LLC, Littleton, MA).

The amplitude of the ERG a- and b-wave was conventionally measured. The amplitude of the a-wave was measured as the voltage difference between the average of the 10 ms prestimulus baseline recorded before the flash and the trough of the a-wave. The b-wave was measured as the voltage difference between the peak of the b-wave and trough of the a-wave.

Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed to determine whether differences in retinal function were significant (P < 0.05). Pairwise multiple comparisons were made using Dunnett’s test when ANOVA detected significant differences. Comparisons between drug treatment groups and vehicle-dosed rats were performed to determine whether retinal protection was significant. Comparison of drug treatment groups with controls allowed assessment of how complete the protection was. An unpaired t-test was used for some comparisons.

Assessment of Retinal Morphology

After a 4-week recovery period from light exposure to allow removal of cellular debris and reorganization of the retina, ocular tissues from drug-treated, vehicle-dosed, and control rats were obtained under deep anesthesia. Orientation of the eye was maintained by placement of a marker suture at the 12-o’clock position before ocular enucleation. The cornea and the lens were then removed, and posterior poles were fixed by immersion in a mixture of paraformaldehyde (2%) and glutaraldehyde (2%) in (0.1 M) phosphate buffer (pH 7.4). Tissues were washed, dehydrated in an ascending ethanol series, and embedded in plastic resin (JB-4; Polysciences, Warrington, PA). Thick sections (1–1.5 μm) were cut and stained (Multiple Stain; Polysciences) and were analyzed using a quantitative computer image analysis system attached to the microscope. Retinal pigment epithelium (RPE), outer nuclear layer (ONL), and inner nuclear layer (INL) thickness as well as inner segment (IS) and outer segment (OS) length were measured to assess outer retina protection. Given that the INL is not significantly affected by light exposure, this layer served as an additional control measurement. Retinal measurements were made at three nasal and three temporal retinal locations separated by 200 μm. For each eye, a minimum of four sections were randomly selected that included one through the optic nerve and three superior retina positions.

ANOVA was performed to determine whether differences in retinal thickness were significant (P < 0.05). The Dunnett’s test was used to perform all pairwise multiple comparisons when ANOVA detected significant differences.

RESULTS

Blue Light Induces Functional Damage to the Retina: Protection by 5-HT₁₄ Agonists

Six-hour blue light exposure induced severe retinopathy in free-moving, vehicle-dosed rats. There was a significant diminution of the ERG response amplitude (ANOVA, P < 0.001) compared with controls when measured after a 5-day recovery (Figs. 1, 2). After data from the three drug evaluation experiments were pooled, the maximum a-wave response amplitude averaged 528 μV ± 5 μV (SEM) from control unexposed rats and 168 μV ± 13 μV (SEM) from light-exposed vehicle-dosed rats (66%–73% decrease). Similarly, maximum b-wave response amplitudes averaged 1345 μV ± 106 μV (SEM) from normal rats and 408 μV ± 29 μV (SEM) from vehicle-dosed rats (67%–71% decrease); a- and b-wave response latencies from light-exposed vehicle-treated rats were increased, and brighter flash intensities were required to elicit threshold responses.

This functional deficit measured 5 days after light exposure was irreversible. When the ERG was reevaluated 3 weeks later, ERG response amplitudes had not recovered: a-wave response amplitudes averaged 569 μV ± 53 μV (SEM) from control unexposed rats and 152 μV ± 21 μV (SEM) from light-exposed vehicle-dosed rats (81%–89% decrease).
amplitudes were reduced by 66%, and b-wave response amplitudes were reduced by 67% (Fig. 2).

**AL-8309A.** AL-8309A is a highly potent, efficacious, and specific agonist of the serotonin 5-HT$_{1A}$ receptor ($EC_{50}$ = 9.6 nM; $E_{\text{max}}$ = 136%). The reported affinity of AL-8309A to other serotonergic, adrenergic, and dopaminergic receptors is 2 to 5 orders of magnitude less than at the 5-HT$_{1A}$ receptor. Five days after light exposure, dose-dependent protection was observed in all rats treated with AL-8309A (Fig. 2A). Significant protection ($P < 0.05$) of retinal function was measured in rats dosed with 0.1 mg/kg AL-8309A compared with vehicle-dosed rats exposed to blue light. Maximum ERG a-wave response amplitudes in AL-8309A (0.1 mg/kg)–treated rats were 60% and b-wave response amplitudes were 63% of normal. Retinal responses from AL-8309A (1.0–30 mg/kg)–dosed rats were not significantly diminished ($P > 0.05$) compared with those of control rats maintained under normal visible cyclic light. A-wave response amplitudes in light-exposed AL-8309A (1–30 mg/kg)–treated rats were between 77% and 89% of normal, and b-wave response amplitudes were 89% to 95% of normal.

After a 4-week recovery period, average ERG response amplitudes from all AL-8309A (0.1–30 mg/kg) treatment groups were significantly higher than those of vehicle-dosed rats and were not statistically distinguishable from those of control rats not exposed to blue light (Fig. 2A). Maximum a-wave response amplitudes were 2.4–3.1-fold higher than in vehicle-dosed rats and 72% to 91% of control amplitudes. Similarly, b-wave response amplitudes were 2.5–3.5-fold higher than responses from vehicle-treated rats and 70% to 94% of control response amplitudes.

**8-OH DPAT.** 8-OH DPAT is the canonical 5-HT$_{1A}$ agonist (binding affinity, 0.58 nM; efficacy, 75.7%). 8-OH DPAT also binds to 5-HT$_{7}$ and a$_{2A}$ receptors, but its binding affinity for the 5-HT$_{1A}$ receptor is approximately 20-fold lower. ERGs recorded from albino rats 5 days after they were exposed to blue light and dosed with 8-OH DPAT (0.1–1.0 mg/kg) were significantly higher than vehicle-dosed rats (Fig. 2B). Response amplitudes from rats dosed with 0.1 and 1.0 mg/kg were approximately twice the amplitude of vehicle-dosed rats. ERG responses were 82% of normal and not statistically different from normal in rats dosed with 0.5 mg/kg 8-OH DPAT. Treatment with 8-OH DPAT (0.05 mg/kg) did not provide significant protection of retinal function compared with blue light exposure in vehicle-dosed rats.

ERGs recorded 4 weeks after blue light exposure in rats dosed with 8-OH DPAT (0.1–1.0 mg/kg) were significantly higher than in vehicle-dosed rats (Fig. 2B). ERG retinal responses recorded in rats dosed with 0.5 mg/kg were approximately 85% of normal. A-wave response amplitudes from 0.5 mg/kg 8-OH DPAT–dosed rats and b-wave response amplitudes from 0.1 and 0.5 mg/kg 8-OH DPAT–dosed rats were not statistically different from normal.

**Buspirone.** Buspirone is a potent partial agonist of the 5-HT$_{1A}$ receptor (binding affinity, 8.9 nM; efficacy, 65.7%). With moderate affinity for brain D$_{2}$ dopamine receptors. ERG response amplitudes measured in buspirone-treated rats 5 days after light exposure demonstrated partial protection of retinal function. ERGs from rats dosed with 0.1 or 1.0 mg/kg buspirone were not significantly higher than from vehicle-dosed rats.
(Fig. 2C). Significant partial protection was measured in rats dosed with 5 and 10 mg/kg buspirone. ERG a-wave response amplitudes were 68% and 56% of normal, and b-wave response amplitudes were 66% of normal in rats dosed with 5 or 10 mg/kg, respectively. In rats dosed with 20 mg/kg buspirone, ERG a-wave response amplitudes were not significantly higher than responses measured in vehicle-dosed rats.

**Blue Light Induces Structural Damage to the Retina: Protection by 5-HT1A Agonists**

Evaluation of retinal lesions 4 weeks after 6-hour blue light exposure demonstrated a significant (ANOVA, \(P < 0.001\)) thinning of the ONL (indicative of photoreceptor cell loss), shortening of their inner and outer segment lengths, and flattening of the RPE in vehicle-dosed animals compared with controls (Fig. 3). Normal RPE thickness (3.1 \(\mu m \pm 0.1 \mu m\) [SEM]), ONL thickness (32 \(\mu m \pm 0.8 \mu m\)), and photoreceptor segment length (23 \(\mu m \pm 0.6 \mu m\)) in control rats not exposed to blue light were reduced 60%, 61%, and 67%, respectively, in vehicle-dosed rats exposed to blue light. No significant INL lesions were detected in rats receiving 6-hour exposure to blue light.

**AL-8309A.** Retinas obtained from rats dosed with AL-8309A (0.1–30 mg/kg) were devoid of significant photic-induced lesions, and retinal thickness measurements were not significantly different from control (Figs. 3C, 3E). RPE thickness varied from 97% to 101% of control and was greater than twofold thicker than RPE thickness measured in vehicle-dosed rats. ONL thickness and inner and outer segment lengths in rats dosed with AL-8309A (0.1 mg/kg) were 85% and 81% of normal, respectively. In rats dosed with AL-8309A (1 or 30 mg/kg), ONL thickness and photoreceptor segment length ranged from 93% to 101% of normal. This degree of retinal preservation is consistent with the functional activity of the retina recorded at this time.

**8-OH DPAT.** Photic lesions in rats dosed with 8-OH DPAT were significantly reduced compared with changes observed in vehicle-dosed rats. 8-OH DPAT–treated retinas demonstrated significantly thicker RPE (0.1–1 mg/kg) and ONL (0.1 and 0.5 mg/kg) layers and significantly longer inner and outer segments (0.1 and 0.5 mg/kg) compared with vehicle-dosed rats (Figs. 3D, 3F). Retinal lesions were not observed in retinas from rats dosed with 0.1 and 0.5 mg/kg 8-OH DPAT, and retinal thickness measurements were not significantly different from those in controls. Less severe retinal lesions were observed in rats dosed with 0.05 mg/kg, but they were not significantly different from vehicle.

**Protection Provided by 5-HT1A Agonists Has a Rapid Onset and Is Persistent**

The experiments described demonstrate that once-daily dosing of rats with 5-HT1A agonists starting 2 days before light exposure and continuing for 2 days afterward provided substantial and statistically significant protection of retinal function in this photo-oxidative induced retinopathy model. Is multiple dosing over several days necessary, or would a single dose of agonist immediately before light exposure suffice to protect? How long does this protective effect last?

To determine the time course of the protection, a single dose of AL-8309A (10 mg/kg) was administered to separate groups of rats 72 hours, 48 hours, 24 hours, or immediately before light exposure to vehicle-dosed rats. Another group received multiple doses according to the standard protocol. The results are shown in Figure 4. ERG a-wave response amplitudes were not significantly higher in rats receiving a single injection of AL-8309A immediately after 6-hour light exposure than in vehicle-dosed rats. In this experiment, comparably protected retinas were measured in rats dosed 24 or 48 hours, but not 72 hours, before light exposure. These data indicate that neuroprotective action of a single dose of AL-8309A is activated rapidly and persists for up to 48 hours.

**Dose Response Protection Provided by a Single Dose of a 5-HT1A Agonist**

The objective of this study was to determine the dose of AL-8309B or 8-OH DPAT required to initiate the cell rescue mechanism and provide approximately 50% protection when administered once, immediately before blue light exposure.
This dose would be used in the 5-HT$_{1A}$ receptor blocking experiment to determine whether cell rescue can be prevented when activation of the 5-HT$_{1A}$ receptor is blocked with a 5-HT$_{1A}$ antagonist. AL-8309A and AL-8309B have the same structure, and both compounds demonstrate similar potency, efficacy, and dose-response curves (data not shown) when compared in the same experiments. AL-8309B was chosen for further evaluation because it was more soluble than AL-8309A.

ERGs recorded from albino rats 5 days after blue light exposure and dosed once with 0.1 mg/kg AL-8309B were not significantly higher than responses recorded from vehicle-dosed rats (Fig. 5A). However, significant partial protection was measured in rats dosed once with AL-8309B (1 and 10 mg/kg) immediately before exposure to blue light. Amplitudes of rats treated with a single dose of 30 mg/kg AL-8309B immediately before exposure to blue light were not significantly different from normal amplitudes. In the 30 mg/kg AL-8309B group, a-wave response amplitudes were 83% of normal and b-wave response amplitudes were 91% of normal. Significant partial protection was also provided by 8-OH DPAT (0.5 mg/kg) administered once immediately before light exposure (Fig. 5B). These data confirm the previous finding that protection is provided rapidly by 5-HT$_{1A}$ agonists. Based on these results, rats were dosed once with 1 mg/kg AL-8309B or 0.5 mg/kg 8-OH DPAT immediately before light exposure in our 5-HT$_{1A}$ receptor blocking experiment with WAY-100635 (0.5 or 5 mg/kg).

5-HT$_{1A}$ Receptor Blockade Prevented 5-HT$_{1A}$ Agonist–Mediated Protection

The objective of this experiment was to determine whether the retinoprotective activity that was measured with 5-HT$_{1A}$ agonists required activation of the 5-HT$_{1A}$ receptor. This was determined by dosing rats with WAY-100635, a highly selective 5-HT$_{1A}$ antagonist, 30 minutes before dosing with AL-8309B or 8-OH DPAT. Treatment of rats with a single dose of AL-8309B (1 mg/kg) or 8-OH DPAT (0.5 mg/kg) immediately before light exposure resulted in significant partial protection of retinal function, and this protection was completely prevented by WAY-100635 (Fig. 6). The antagonist alone neither exacerbated damage nor provided protection from it. This is strong evidence that the neuroprotection afforded by AL-8309B and 8-OH DPAT is caused by agonism at the 5-HT$_{1A}$ receptor.

Topical Ocular Dosing of 1.75% AL-8309B Is Retinoprotective

Topical ocular dosing once or twice daily for 21 days resulted in significant protection of retinal function in rats exposed to a blue light insult for 6 hours (Fig. 7). Response amplitudes of both a- and b-wave amplitudes in both dosing groups were significantly higher than responses recorded in vehicle-dosed rats.

DISCUSSION

The present study provides the first evidence that photic-induced retinal degeneration can be prevented or suppressed by treatment with 5-HT$_{1A}$ receptor agonists. Acute blue light exposure resulted in severe, irreversible retinal lesions. Five days after light exposure, the ERG was reduced approximately 69%; when reevaluated 3 weeks later, there was no recovery of ERG response amplitudes. RPE cells were flattened, photoreceptor cell loss was obvious, and photoreceptor inner and outer segment lengths were shortened. 8-OH DPAT (0.5–10 mg/kg),$^{31,33,34,37,38}$ the most potent 5-HT$_{1A}$ receptor full agonist tested, and buspirone (10 mg/kg),$^{43,46}$ a partial 5-HT$_{1A}$ agonist, demonstrated neuroprotective activity in animal CNS injury models. In vivo neuroprotective activity of AL-8309A has not been reported, although it was shown to improve the recovery of spinal action potential amplitudes in an ex vivo spinal cord compression injury model. In the blue light phototoxicity model, all 5-HT$_{1A}$ agonists tested provided dose-dependent protection of the ERG and retinal morphology.

**FIGURE 4.** Rats were dosed (subcutaneously) with AL-8309A (10 mg/kg) once at various times before, during, or after 6-hour exposure to blue light. For comparison, one group of light-exposed rats was dosed using our standard dosing schedule. ERGs from rats dosed once 48 hours, 24 hours, or immediately before light exposure or using our standard dosing schedule were significantly higher ($P < 0.05$) than vehicle-dosed rats and not statistically distinguishable ($P > 0.05$) from rats not exposed to blue light (NL). Treatment of rats with AL-8309A after light exposure was not protective. The neuroprotective action of a single dose of AL-8309A has a rapid onset and persists for 48 hours. Each treatment group consisted of 10 rats.

**FIGURE 5.** Rats were treated (subcutaneously) with vehicle or a 5-HT$_{1A}$ agonist once, immediately before light exposure. ERGs were compared in the same experiments. AL-8309B was chosen for further evaluation because it was more soluble than AL-8309A. Each treatment group consisted of 10 rats; six control rats were evaluated. These data indicate that protection by 5-HT$_{1A}$ agonists has a rapid onset.
Buspirone and 8-OH DPAT had potency similar to that seen in CNS injury models. Treatment with AL-8309A (1–30 mg/kg) and 8-OH DPAT (0.5 mg/kg) starting 2 days before light exposure prevented blue light–induced functional and structural retinal lesions. The advantage of AL-8309A was that it provided complete protection over a 30-fold concentration range, whereas 8-OH DPAT exhibited an inverted dose-response curve, providing only partial protection at doses above or below 0.5 mg/kg. AL-8309B also showed a similar broad range of concentrations that provided complete protection (data not shown). Buspirone was the least potent and efficacious, providing only partial protection at doses between 5 and 20 mg/kg.

Light damage models have been used to evaluate a number of therapeutic interventions that may have usefulness in the treatment of retinal degenerative disease. Compounds that minimized retinal photo-oxidative injury include antioxidants such as ascorbate,47,48 dimethylthiourea,49–52free-radical spin trapping agents such as phenyl-N-tert-butylnitrone,53 thioredoxin,54 and superoxide dismutase (SOD) mimics55,56; the calcium antagonist flunarizine 57,58; caspase inhibitors 59,60; slowing rhodopsin regeneration with 13-cis retinoic acid 61; and neurotrophic factors62–64or drugs that upregulate endogenous retinal trophic factors such as /H92512-agonists65 and /H9252-blockers.44 With the exception of trophic factors, thioredoxin, and caspase inhibitors, which were injected into the vitreous cavity, comparing published efficacy data for neuroprotective agents in light damage models to those reported here, AL-8309A was 5-fold more potent than clonidine (2-agonist),65 60-fold more potent than xylazine (2-agonist),65 and greater than 200-fold more potent than the other compounds evaluated.44,48,50,53,55,57,58,61,65 For example, Agarwal 44 demonstrated efficacy in a light-damage model with 20 mg/kg levobetaxolol (2-blocker). Compared with AL-8309A (0.1 mg/kg), levobetaxolol was 200 times less potent. Potency, favorable neuroprotective efficacy, and broad dose-response curve measured in this model differentiated AL-8309A from other 5-HT1A agonists.

In the CNS, the conclusion that the effects of 5-HT1A agonists are mediated by 5-HT1A receptors has been supported by multiple studies with WAY-100635,40,42,66 an antagonist with greater than 100-fold selectivity for 5-HT1A receptors relative to binding for other serotoninergic, adrenergic, or dopaminergic receptors.68 To determine whether retinal neuroprotection measured in rats treated with 5-HT1A agonists requires activation of the 5-HT1A receptor, rats were predosed with WAY-100635 before they were dosed with AL-8309B or 8-OH DPAT. Pretreatment with WAY-100635 potently and dose-dependently blocked the retinoprotective activity measured with 5-HT1A agonists that is caused by activation of the 5-HT1A receptor. Each treatment group consisted of 10 to 20 rats.

FIGURE 6. To determine whether the retinoprotective activity measured with 5-HT1A agonists requires activation of the 5-HT1A receptor, rats were dosed (subcutaneously) with a selective 5-HT1A antagonist, WAY-100635, 30 minutes before light exposure and with the 5-HT1A agonist (A) AL-8309B or (B) 8-OH DPAT immediately before light exposure. ERGs were evaluated 5 days after a 6-hour exposure to blue light. Maximum a- and b-wave response amplitudes were significantly reduced in vehicle-dosed rats exposed to blue light compared with rats not exposed to blue light (NL). *Treatment with 5-HT1A agonist (A) AL-8309B (1 mg/kg) or (B) 8-OH DPAT (0.5 mg/kg) provided significant partial protection (P < 0.05). Protection provided by (A) AL-8309B was blocked by pretreatment with WAY-100635 (1 and 10 mg/kg). Similarly, protection provided by (B) 8-OH DPAT was blocked by pretreatment with WAY-100635 (0.5 and 5 mg/kg). Treatment with WAY-100635 (0.5, 5, or 10 mg/kg) alone neither exacerbated light damage nor provided protection from it. ERGs of rats dosed with a 5-HT1A agonist or WAY-100635 and not exposed to blue light were not different from those of controls. Neuroprotection afforded by 5-HT1A agonists tested was caused by activation of the 5-HT1A receptor. Each treatment group consisted of 10 to 20 rats.

FIGURE 7. ERGs from rats exposed to blue light that received topical ocular dosing of AL-8309B (1.75%) once a day or twice daily were significantly higher (*P < 0.05) than responses recorded in vehicle-dosed rats. Each treatment group consisted of 20 to 24 rats.
dependently blocked the neuroprotective effect of AL-8309B and 8-OH DPAT in this severe photo-oxidative stress model. WAY-100635 had no effect on normal retinal function and did not enhance or prevent the damaging effects of blue light on the retina. Therefore, retinal protection afforded by the 5-HT1A agonists tested is caused by activation of the 5-HT1A receptor.

The mechanisms underlying the neuroprotective properties of 5-HT1A agonists in the CNS are not completely understood. Several putative mechanisms that have been identified include neuronal membrane hyperpolarization35,65 mediated by G protein–coupled K+ channels,79 decreasing glutamate release,71 and blockade of Ca2+ channels2 or Na+ channels.75 Recently, there has been increasing evidence implicating 5-HT1A receptors activating the mitogen-activated protein kinase (MAPK/ERK) signaling pathway, influencing gene expression and leading to cell survival.74,75 This gene regulation can lead to increased expression of several components of the antioxidant defense system, including SOD and catalase.43,75,76 antiapoptotic proteins from the prosurvival members of the BCL family (e.g., Bcl-2 and Bcl-XL),32,77 and inhibitors of apoptosis proteins (e.g., XIAP).32,77,78 In addition, 5-HT1A receptor activation of ERK results in an inhibition of caspase 3.74 We have shown that incubation of ARPE-19 cells with AL-8309A leads to an increase of ERK 1/2 phosphorylation and to subsequent upregulation of antioxidant and antiapoptotic proteins, including SOD-1, SOD-2, Bcl-2, and Bcl-XL (Rhoades KL, et al. IOVS 2009;50:ARVO E-Abstract 677). Other potential mechanisms include increased expression of brain-derived neurotrophic factor mRNA,79 S100B,20 and nerve growth factor.18,19 The precise molecular mechanisms involved in 5-HT1A receptor-mediated neuroprotection remain to be defined. The exploration of molecular events between 5-HT1A receptors and MEK activation, and between ERK activation and antiapoptotic and antioxidant protein upregulation, will provide important insights into cell survival signaling in the retina.

When dosed once immediately before light exposure, AL-8309A and AL-8309B in sufficient doses provided complete protection. In these studies, 5-HT1A-mediated neuroprotective mechanisms were activated rapidly and persisted for up to 2 days. Activation of the MAPK/ERK signaling pathway occurs quickly in the CNS and can lead to rapid upregulation of protein expression. Crane57 has shown that 5 minutes after injection of 8-OH DPAT (0.2 mg/kg), phospho-ERK levels increased in the hypothalamic paraventricular nucleus and medial basal hypothalamus. In studies reported by Sullivan,75 tandospirone (10 mg/kg) led to increased phospho-ERK levels in the hypothalamic paraventricular nucleus and dorsolateral nucleus 5 minutes after injection, and plasma levels of ACTH and oxytocin peaked by 15 minutes. Studies are ongoing to determine the temporal relationship between administration of 5-HT1A agonist activation of signal transduction pathways and upregulation of antioxidant and antiapoptotic defenses. Dosing after light exposure was too late to be effective, probably because of the activation of cell death pathways during light exposure. Immediately after light exposure, we observed that photoreceptor segment length was significantly shortened and that approximately 40% to 50% of the photoreceptor nuclei were pyknotic.

The 5-HT1A receptor agonist AL-8309B may offer a novel approach to retinal neuroprotection for outer retinal disorders such as atrophic AMD. Oxidative stress and inflammation are thought to play pivotal roles in the etiology of AMD.80–82 Oxidation products of lipids, nucleic acids, and proteins are abundantly present in eyes of patients with geographic atrophy.83 Crabbe34 has identified a number of oxidized proteins and protein adducts in drusen, including CEP. Autoantibodies to CEP in the plasma,84,85 CEP adducts in drusen, and CEP-adducted proteins are more abundant in AMD patients than in age-matched controls. Mice immunized with CEP adducts accumulate drusen-like deposits on Bruch’s membrane and develop RPE lesions similar to those observed in patients with geographic atrophy.86 SOD2-knockdown mice exhibit increased autofluorescence, elevated bis-retinaldehyde-phosphatidylethanolamine (A2E) levels, CEP adducts, Bruch’s membrane thickening, RPE degeneration, and ONL thickening.87

AL-8309A was shown to upregulate antioxidant defense mechanisms in the retina (Rhoades KL, et al. IOVS 2009;50:ARVO E-Abstract 677) and to prevent CEP adduct formation (Renganathan K, et al. IOVS 2009;50:ARVO E-Abstract 681) and deposition of complement C3, factor B, factor F, and membrane attack complex (Wang Y, et al. IOVS 2009;50:ARVO E-Abstract 685). Topical ocular dosing with 1.75% AL-8309B once or twice daily provided protection from photo-oxidative damage to the retina. This is an attractive route of administration for human studies evaluating potential therapeutics to treat retinal disorders. A clinical trial88 evaluating the safety and efficacy of topically administered AL-8309B for the treatment of advanced nonexudative AMD (geographic atrophy) is under way.

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