

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978**PUBLICATION PARTICULARS AND ABSTRACT**

(Section 32(3)(a) – Regulation 22(1)(g) and 31)

OFFICIAL APPLICATION NO.

LODGING DATE

ACCEPTANCE DATE

21 01 2002/ 6350 -

22 8 AUG 2002

43 8.8.03

INTERNATIONAL CLASSIFICATION

NOT FOR PUBLICATION

51 A61K ; A61P

CLASSIFIED BY: WIPO

FULL NAMES OF APPLICANT

71 ALCON, INC.

FULL NAMES OF INVENTORS

72 1. COLLIER JR., ROBERT J
2. KAPIN, MICHAEL A
3. HELLBERG, MARK R
4. DEAN, THOMAS R

EARLIEST PRIORITY CLAIMED

COUNTRY

NUMBER

DATE

33 US

31 60/190,279

32 17 MAR 2000

TITLE OF INVENTION

54 COMPOUNDS WITH 5-HT_{1A} ACTIVITY USEFUL FOR TREATING DISORDERS OF THE OUTER RETINA

57 ABSTRACT (NOT MORE THAT 150 WORDS)

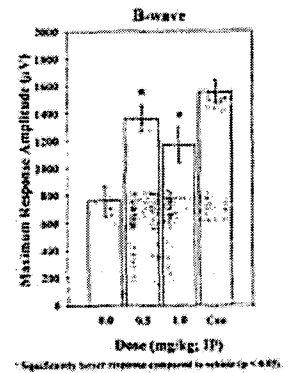
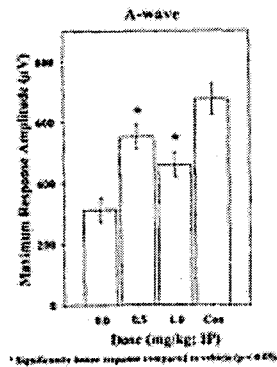
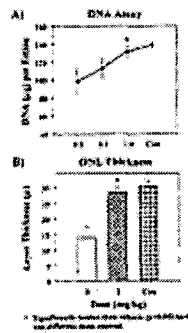
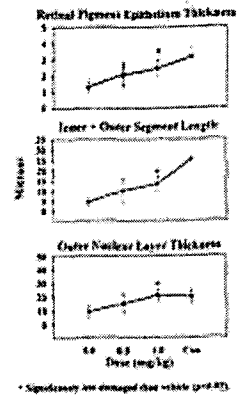
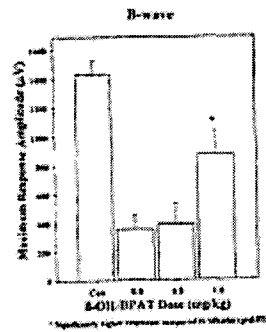
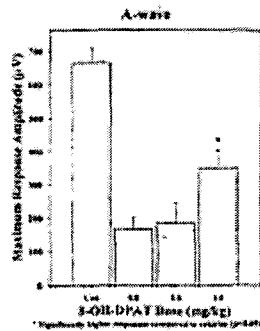
NUMBER OF SHEETS

37

If no classification is finished, Form P.9 should accompany this form.
The figure of the drawing to which the abstract refers is attached.

Abstract

Compositions and methods for treating disorders of the outer retina with compounds with 5-HT_{1A} agonist activity are disclosed.



COMPOUNDS WITH 5-HT_{1A} ACTIVITY USEFUL FOR TREATING DISORDERS OF THE OUTER RETINA

The present invention is directed to compounds with 5-HT_{1A} agonist activity useful for treating disorders of the outer retina resulting from acute or chronic degenerative conditions or diseases of the eye.

Background of the Invention

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, with an incidence of about 20% in adults 65 years of age increasing to 37% in individuals 75 years or older. Non-exudative AMD is characterized by drusen accumulation and atrophy of rod and cone photoreceptors in the outer retina, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaris; while exudative AMD leads to choroidal neovascularization (Green and Enger, *Ophthalmol*, 100:1519-35, 1993; Green et al., *Ophthalmol*, 92:615-27, 1985; Green and Key, *Trans Am Ophthalmol Soc*, 75:180-254, 1977; Bressler et al., *Retina*, 14:130-42, 1994; Schneider et al., *Retina*, 18:242-50, 1998; Green and Kuchle (1997). In: Yannuzzi, L.A., Flower, R.W., Slakter, J.S. (Eds.) *Indocyanine green angiography*. St. Louis: Mosby, p. 151-6). Retinitis pigmentosa (RP) represents a group of hereditary dystrophies characterized by rod degeneration with secondary atrophy of cone photoreceptors and underlying pigment epithelium. (Pruett, *Trans Am Ophthalmol Soc*, 81:693-735, 1983; Heckenlively, *Trans Am Ophthalmol Soc*, 85:438-470, 1987; Pagon, *Sur Ophthalmol*, 33:137-177, 1988; Berson, *Invest Ophthalmol Vis Sci*, 34:1659-1676, 1993; Nickells and Zack, *Ophthalmic Genet*, 17:145-65, 1996). The pathogenesis of retinal degenerative diseases such as AMD and RP is multifaceted and can be triggered by environmental factors in normal individuals or in those who are genetically predisposed. To date more than 100 genes have been mapped or cloned that may be associated with various outer retinal degenerations.

Light exposure is an environmental factor that has been identified as a contributing factor to the progression of retinal degenerative disorders such as AMD (Young, *Sur Ophthalmol*, 32:252-269, 1988; Taylor, et al., *Arch Ophthalmol*, 110:99-104, 1992; Cruickshank, et al., *Arch Ophthalmol*, 111:514-518, 1993). Photo-oxidative stress leading to light damage to retinal cells has been shown to be a useful model for studying retinal degenerative diseases for the following reasons: damage is primarily to the photoreceptors and retinal pigment epithelium (RPE) of the outer retina, the

same cells that are affected in heredodegenerative diseases (Noell et al., *Invest Ophthalm Vis Sci*, 5, 450-472, 1966; Bressler et al., *Sur Ophthalm*, 32, 375-413, 1988; Curcio et al., *Invest Ophthalm Vis Sci*, 37, 1236-1249, 1996); apoptosis is the cell death mechanism by which photoreceptor and RPE cells are lost in AMD and RP, as well as following a photo-oxidative induced cell injury (Ge-Zhi et al., *Trans AM Ophthalm Soc*, 94, 411-430, 1996; Abler et al., *Res Commun Mol Pathol Pharmacol*, 92, 177-189, 1996; Nickells and Zack, *Ophthalmic Genet*, 17:145-65, 1996); light has been implicated as an environmental risk factor for progression of AMD and RP (Taylor et al., *Arch Ophthalmol*, 110, 99-104, 1992; Naash et al., *Invest Ophthalm Vis Sci*, 37, 775-782, 1996); and therapeutic interventions which inhibit photo-oxidative injury have also been shown to be effective in animal models of heredodegenerative retinal disease (LaVail et al., *Proc Nat Acad Sci*, 89, 11249-11253, 1992; Fakforovich et al., *Nature*, 347, 83-86, 1990; Frasson et al., *Nat. Med.* 5, 1183-1187, 1990).

A number of different compound classes have been identified in various animal models that minimize retinal photo-oxidative injury. They include: antioxidants such as ascorbate (Organisciak et al., *Invest Ophthalm Vis Sci*, 26:1589-1598, 1985), dimethylthiourea (Organisciak et al., *Invest Ophthalm Vis Sci*, 33:1599-1609, 1992; Lam et al., *Arch Ophthalm*, 108:1751-1752, 1990), α -tocopherol (Kozaki et al., *Nippon Ganka Gakkai Zasshi*, 98:948-954, 1994) and β -carotene (Rapp et al., *Cur Eye Res*, 15:219-232, 1995); calcium antagonists such as flunarizine (Li et al., *Exp Eye Res*, 56: 71-78, 1993; Edward et al., *Arch Ophthalm*, 109, 554-622, 1992; Collier et al., *Invest Ophthalm Vis Sci*, 36:S516); growth factors such as basic-fibroblast growth factor, brain derived nerve factor, ciliary neurotrophic factor, and interleukin-1- β (LaVail et al., *Proc Nat Acad Sci*, 89, 11249-11253, 1992); glucocorticoids such as methylprednisolone (Lam et al., *Graefes Arch Clin Exp Ophthalm*, 231, 729-736, 1993) and dexamethasone (Fu et al., *Exp Eye Res*, 54, 583-594, 1992); iron chelators such as desferrioxamine (Li et al., *Cur Eye Res*, 2, 133-144, 1991); NMDA-antagonists such as eliprodil and MK-801 (Collier et al., *Invest Ophthalm Vis Sci*, 40:S159, 1999).

Serotonergic 5-HT_{1A} agonists (i.e., buspirone, ziprasidone, urapidil) have either been registered or launched for the treatment of anxiety, hypertension, schidzophrenia, psychosis or depression-bipolar disorders. In addition, 5-HT_{1A} agonists have been shown to be neuroprotective in various animal models and are being evaluated in the clinic to treat cerebral ischemia, head trauma, Alzheimer's Disease, Multiple Sclerosis and amyotrophic lateral sclerosis. The 5-HT_{1A} agonists, 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) and ipsapirone, were shown to

prevent NMDA-induced excitotoxic neuronal damage in the rat magnocellular nucleus
basalis (Oosterink et al., *Eur J Pharmacol*, 358:147-52, 1998), dosing with Bay-x-
3702 significantly reduced ischemic damage in a rat acute subdermal hematoma
model (Alessandri et al., *Brain Res*, 845:232-5, 1999), while 8-OH-DPAT, Bay-x-
3702, urapidil, gepirone and CM 57493 significantly reduced cortical infarct volume
in the rat (Bielenberg and Burkhardt, *Stroke*, 21(Suppl): IV161-3; Semkova et al., *Eur*
J Pharmacol, 359:251-60, 1998; Peruche et al., *J Neural Transm – Park Dis Dement*
Sect, 8:73-83, 1994) and mouse (Prehn et al., *Eur J Pharmacol*, 203:213-22, 1991;
Prehn et al., *Brain Res*, 630:10-20, 1993) after occlusion of the middle cerebral artery.
In addition, treatment of rats with SR 57746A, a potent 5-HT_{1A} agonist, has been
shown to be neuroprotective following 4-vessel transient global ischemia, vincristine
sulphate induced septohippocampal lesions, acrylamide-induced peripheral
neuropathy, and sciatic nerve crush (Fournier et al., *Neurosci*, 55:629-41, 1993) and
has been shown to delay the progression of motor neuron degeneration in *pnn* mice
(Fournier et al., *Br J Pharmacol*, 124:811-7, 1998).

This class of compounds has been disclosed for the treatment of glaucoma
(lowering and controlling IOP), see e.g., WO 98/18458 (DeSantis, et al) and EP
0771563A2 (Mano, et al.). Osborne, et al. (*Ophthalmologica*, Vol. 210:308-314,
1996) teach that 8-hydroxydipropylaminotetralin (8-OH-DPAT) (a 5-HT_{1A} agonist)
reduces IOP in rabbits. Wang, et al. (*Current Eye Research*, Vol. 16(8):769-775,
August 1997, and *IVOS*, Vol. 39(4), S488, March, 1998) disclose that 5-
methylurapidil, an α_{1A} antagonist and 5-HT_{1A} agonist lowers IOP in the monkey, but
due to its α_{1A} receptor activity. Also, 5-HT_{1A} antagonists are disclosed as being useful
for the treatment of glaucoma (elevated IOP) (e.g. WO 92/0338, McLees).
Furthermore, DeSai, et al. (WO 97/35579) and Macor, et al. (U.S. 5,578,612) disclose
the use of 5-HT₁ and 5-HT_{1-like} agonists for the treatment of glaucoma (elevated IOP).
These anti-migraine compounds are 5-HT_{1B,D,E,F} agonists, e.g., sumatriptan and
naratriptan and related compounds.

Brief Description of the Drawings

Figures 1A and 1B show the preservation of the ERG a- and b-wave function in rats
dosed systemically with 8-OH-DPAT and exposed to a severe photo-oxidative insult.

Figure 2 shows protection of retinal morphology (photoreceptors and RPE) in rats
dosed systemically with 8-OH-DPAT and exposed to a severe photo-oxidative insult.

Figure 3 shows protection of retinal DNA, a measure of retinal cell number (A), and complete protection of retinal morphology (photoreceptors) in rats dosed systemically with buspirone and exposed to a severe photo-oxidative insult.

Figure 4A and 4B show the preservation of the ERG a- and b-wave function in rats dosed systemically with SR-57746A and exposed to a severe photo-oxidative insult.

Summary of the Invention

The present invention is directed to 5-HT_{1A} agonists which have been discovered to be useful in treating disorders of the outer retina, particularly: AMD; RP and other forms of hereditary degenerative retinal disease; retinal detachment and tears; macular pucker; ischemia affecting the outer retina; diabetic retinopathy; damage associated with laser therapy (grid, focal, and panretinal) including photodynamic therapy (PDT); trauma; surgical (retinal translocation, subretinal surgery, or vitrectomy) or light-induced iatrogenic retinopathy; and preservation of retinal transplants.

Description of Preferred Embodiments

Serotonergic 5-HT_{1A} agonists have been shown to be potent neuroprotective agents following varying insults to the central nervous system. Unexpectedly, we have demonstrated that 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), buspirone and SR-57746A exhibit potent neuroprotective activity in the retina and prevent light-induced apoptotic cell death to photoreceptors and RPE cells. We have found that treatment with buspirone can completely prevent photo-oxidative induced retinopathy and significantly reduce loss of retinal DNA and ONL thinning. The safety advantages of some of these compounds make them particularly desirable for both acute and chronic therapies. Such an agent would have utility in the treatment of various outer retinal degenerative diseases.

In our light damage paradigms, antioxidants were either ineffective (α -tocopherol) or marginally effective at high doses (ascorbate, vitamin E analogs). Similarly, some calcium antagonists (flunarizine, nifedipine) were moderately effective while others (nifedipine, nimodipine, verapamil) had no effect in preventing light-induced functional or morphological changes. However, it has been discovered that 5-HT_{1A} agonists are 100-fold more potent in these light damage paradigms and therefore are useful for treating disorders of the outer retina.

The invention contemplates the use of any pharmaceutically acceptable 5-HT_{1A} agonist, including pharmaceutically acceptable salts, for treating disorders of the outer retina (Compounds). Pharmaceutically acceptable means the Compounds can be safely used for the treatment of diseases of the outer retina. As used herein, the outer retina includes the RPE, photoreceptors, Muller cells (to the extent that their processes extend into the outer retina), and the outer plexiform layer. The compounds are formulated for systemic or local ocular delivery.

Disorders of the outer retina encompass acute and chronic environmentally induced (trauma, ischemia, photo-oxidative stress) degenerative conditions of the photoreceptors and RPE cells in normal or genetically predisposed individuals. This would include, but not limited to, AMD, RP and other forms of heredodegenerative retinal disease, retinal detachment, tears, macular pucker, ischemia affecting the outer retina, diabetic retinopathy, damage associated with laser therapy (grid, focal and panretinal) including photodynamic therapy (PDT), thermal or cryotherapy, trauma, surgical (retinal translocation, subretinal surgery or vitrectomy) or light induced iatrogenic retinopathy and preservation of retinal transplants.

Compounds of the present invention have potent affinity for 5-HT_{1A} receptors with IC₅₀ values that range up to about 500 nM (preferably less than 100 nM). These Compounds are also either full or partial agonists with IC₅₀ values ranging up to about 1 μ M (preferably less than 500 nM). Representative 5-HT_{1A} agonists useful according to the present invention include, but are not limited to: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, Org-13011, Org-12966, E-5842, SUN-N4057, and 8-OH-DPAT.

Receptor binding and agonist activity according to this invention can be determined using the following methods.

METHOD 1

5-HT_{1A} Receptor Binding Assay

5-HT_{1A} binding studies were performed with human cloned receptors expressed in Chinese hamster ovary (CHO) cells using (³H)8-OH DPAT as the ligand. Membranes from Chinese hamster ovary cells (CHO) expressing cloned 5-HT_{1A} receptors (manufactured for NEN by Biosignal, Inc., Montreal, Canada) were homogenized in approximately 40 volumes of 50 mM Tris pH 7.4 for 5 sec. Drug dilutions were made using a Beckman Biomek 2000 robot (Beckman Instruments, Fullerton, CA). Incubations were conducted with membrane prep, test compounds, and 0.25 nM [³H]8-OH-DPAT (NEN, Boston, MA) in the same buffer at 27°C for 1 h. Assays were terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters pre-soaked in 0.3% polyethyleneimine. Bound radioactivity was measured using liquid scintillation spectrometry. Data were analyzed using non-linear curve fitting programs (Sharif et al., J Pharmac Pharmacol, 51: 685-694, 1999).

Ligand binding studies can also be run using membrane preparations from calf and rat brain (local source) and human cortex membranes. Specific brain regions were dissected out, homogenized in 10 volumes of 0.32 M sucrose and centrifuged for 10 min at 700 x g. The resulting supernatant was centrifuged at 43,500 x g for 10 min and the pellet re-suspended in 50 mM Tris-HCl (pH 7.7, 25°C) using a 10 sec polytron treatment. Aliquots were stored at -140° C. To remove endogenous serotonin, the preps were incubated at 37° C for 10 min prior to the experiment. Assay incubations were terminated by rapid filtration over Whatman GF/C filters using a Brandel cell harvester. K_i values were calculated using the Cheng-Prusoff equation (De Vry et al., J Pharm Exper Ther, 284:1082-1094, 1998.)

METHOD 2

5-HT_{1A} Functional Assays

The function of Compounds of the present invention can be determined using a variety of methods to assess the functional activity of 5-HT_{1A} agonists. One such assay is performed using hippocampal slices from male Sprague-Dawley rats, measuring the inhibition of forskolin-stimulated adenylate cyclase (J Med Chem, 42:36, 1999; J Neurochem, 56:1114, 1991; J Pharm Exper Ther, 284:1082, 1998). Rat hippocampal membranes were homogenized in 25 volumes of 0.3 M sucrose containing 1mM EGTA, 5 mM EDTA, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 7.4 at 25°C. The homogenate was centrifuged for 10 min at 1,000 x g. The

supernatant subsequently was centrifuged at 39,000 x g for 10 min. The resulting pellet was re-suspended in homogenization buffer at a protein concentration of approximately 1 mg/ml and aliquots were stored at -140°C. Prior to use, the membranes were rehomogenized in a Potter-Elvehjem homogenizer. Fifty µl of the membrane suspension (50 µg protein) were added to an incubation buffer containing 100 mM NaCl, 2 mM magnesium acetate, 0.2 mM ATP, 1 mM cAMP, 0.01 mM GTP, 0.01 mM forskolin, 80 mM Tris-HCl, 5 mM creatine phosphate, 0.8 U/µl creatine phosphokinase, 0.1 mM IBMX, 1-2 µCi α-[³²P]ATP. Incubations with test compounds (10 min at 30°C) were initiated by the addition of the membrane solution to the incubation mixture (prewarmed 5 min at 30°C). [³²P]cAMP was measured according to the method of Salomon (Adv Cyclic Nucleotide Res, 10:35-55, 1979). Protein was measured using the Bradford (Anal Biochem, 72:248-254, 1976) assay.

Functional activity can also be determined in recombinant human receptors according to the method of Schoeffer et al. (Neuropharm, 36:429-437, 1997). HeLa cells transfected with recombinant human 5-HT_{1A} receptors were grown to confluence in 24-well plates. The cells were rinsed with 1 ml of Hepes-buffered saline (in mM) NaCl 130, KCl 5.4, CaCl₂ 1.8, MgSO₄ 0.8, NaH₂PO₄ 0.9, glucose 25, Hepes 20, pH 7.4, and phenol red 5 mg/l. The cells were labelled with 6 µCi/ml of [³H]adenine (23 Ci/mmol, Amersham, Rahn AG, Zurich, Switzerland) in 0.5 ml of saline at 37 °C for 2 hr. The plates were subsequently rinsed twice with 1 ml of buffered saline containing 1mM isobutylmethylxanthine. The cells were incubated for 15 min in 1 ml of this solution (37 °C) in the presence or absence of 10 µM forskolin and the test compound. The buffer was then removed and 1 ml of 5% trichloroacetic acid (TCA) containing 0.1 mM cAMP and 0.1 mM ATP was added to extract the samples. After 30 min at 4°C, the TCA extracts were subjected to chromatographic separation on Dowex AG 50W-X4 and alumina columns (Salomon, Meth Enzymol, 195: 22-28, 1991). Cyclic AMP production was calculated as the ratio [³H]cAMP/([³H]cAMP + [³H]ATP).

The above procedures described in Methods 1 and 2 were used to generate the following data.

Table 1. 5-HT_{1A} Receptor Binding and Functional Assay Data.

Compound	Receptor Binding (IC₅₀ nM, SEM)	cAMP Inhibition (EC₅₀)
(R,S) 8-OH-DPAT	1.5 nM	4.7 nM
(R) 8-OH-DPAT	0.5 nM	2.6 nM
SR-57746A	2.5 nM	3.7 nM

In general, for degenerative diseases, the 5-HT_{1A} agonists of this invention are administered orally with daily dosage of these compounds ranging between about 0.001 and about 500 milligrams. The preferred total daily dose ranges between about 1 and about 100 milligrams. Non-oral administration, such as, intravitreal, topical ocular, transdermal patch, subdermal, parenteral, intraocular, subconjunctival, or retrobulbar or subtenon's injection, trans scleral (including iontophoresis), or slow release biodegradable polymers or liposomes may require an adjustment of the total daily dose necessary to provide a therapeutically effective amount of the compound. The 5-HT_{1A} agonists can also be delivered in ocular irrigating solutions. Concentrations should range from about 0.001 μ M to about 100 μ M, preferably about 0.01 μ M to about 5 μ M.

The 5-HT_{1A} agonists can be incorporated into various types of ophthalmic formulations for delivery to the eye (e.g., topically, intracamerally, or via an implant). They may be combined with ophthalmologically acceptable preservatives, surfactants, viscosity enhancers, gelling agents, penetration enhancers, buffers, sodium chloride, and water to form aqueous, sterile ophthalmic suspensions or solutions or preformed gels or gels formed in situ. Ophthalmic solution formulations may be prepared by dissolving the compound in a physiologically acceptable isotonic aqueous buffer. Further, the ophthalmic solution may include an ophthalmologically acceptable surfactant to assist in dissolving the compound. The ophthalmic solutions may contain a viscosity enhancer, such as, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, methylcellulose, polyvinyl-pyrrolidone, or the like, to improve the retention of the formulation in the conjunctival sac. In order to prepare sterile ophthalmic ointment formulations, the active ingredient is combined with a preservative in an appropriate vehicle, such as, mineral oil, liquid lanolin, or white petrolatum. Sterile ophthalmic gel formulations may be prepared by suspending the

active ingredient in a hydrophilic base prepared from the combination of, for example, carbopol-940, or the like, according to the published formulations for analogous ophthalmic preparations; preservatives and tonicity agents can be incorporated.

5 If dosed topically, the 5-HT_{1A} agonists are preferably formulated as topical ophthalmic suspensions or solutions, with a pH of about 4 to 8. The 5-HT_{1A} agonists will normally be contained in these formulations in an amount .001% to 5% by weight, but preferably in an amount of .01% to 2% by weight. Thus, for topical presentation, 1 to 2 drops of these formulations would be delivered to the surface of
10 the eye 1 to 4 times per day according to the discretion of a skilled clinician.

The following topical ophthalmic formulations are useful according to the present invention administered 1-4 times per day according to the discretion of a skilled clinician.

EXAMPLE 1

Ingredients	Amount (wt %)
Buspirone	0.01 – 2%
Hydroxypropyl methylcellulose	0.5%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 2

Ingredients	Amount (wt %)
Buspirone	0.01 – 2%
Methyl cellulose	4.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 3

Ingredients	Amount (wt %)
Compound	0.01 – 2%
Guar gum	0.4- 6.0%
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4
Purified water	q.s. to 100%

EXAMPLE 4

Ingredients	Amount (wt %)
Xaliprodén hydrochloride	0.01 – 2%
White petrolatum and mineral oil and lanolin	Ointment consistency
Dibasic sodium phosphate (anhydrous)	0.2%
Sodium chloride	0.5%
Disodium EDTA (Edetate disodium)	0.01%
Polysorbate 80	0.05%
Benzalkonium chloride	0.01%
Sodium hydroxide / Hydrochloric acid	For adjusting pH to 7.3 – 7.4

5

EXAMPLE 5

10mM IV Solution w/v%	
Buspirone	0.384%
L-Tartaric acid	2.31%
Sodium hydroxide	pH 3.8
Hydrochloric acid	pH 3.8
Purified water	q.s. 100%

EXAMPLE 6

5mg Capsules	
Ingredient	mg/capsule (Total Wt. 22a mg)
Buspirone Hydrochloride	5
Lactose, anhydrous	55.7
Strach, Sodium carboxy-methyl	8
Cellulose, microcrystalline	30
Colloidal silicon dioxide	.5
Magnesium stearate	.8

METHOD 3**Neuroprotective effects in the rat photo-oxidative induced retinopathy model**

The retinal protective effect of these 5-HT_{1A} agonists were evaluated in our photo-oxidative induced retinopathy paradigm.

Induction of Photochemical Lesion. Photochemical lesions were induced in dark adapted rats (24 hour) by exposure to (220 fc) blue light (half-amplitude bandpass = 435-475 nm) for 6 hours. Animals were allowed to recover for 5 days in darkness prior to electrodiagnostic evaluation of retinal function. Rats were single housed in clear polycarbonate cages during this light exposure.

Electrodiagnostic Evaluation. The electroretinogram (ERG) was recorded from anesthetized rats after a 24-hour dark-adaptation period. Rats were anesthetized by IP injection with Ketamine-HCl (75 mg/Kg) and Xylazine (6 mg/Kg). Flash ERGs recorded from a platinum-iridium wire loop electrode positioned on the cornea were elicited by viewing a ganzfeld. Electrical responses to a series of light flashes increasing in intensity were digitized to analyze temporal characteristics of the waveform and response voltage-log intensity (VlogI) relationship. Changes in the ERG a-wave are associated with photoreceptor and retinal pigment epithelium damage while damage to the inner retina is reflected in changes in the ERG b-wave.

Assessment of Retinal Morphology. Ocular tissues were obtained from control and drug or vehicle dosed rats and fixed by immersion into a mixture of 2%

paraformaldehyde and 2% glutaraldehyde. Fixed eyeballs were dehydrated in an ascending ethanol series, embedded in JB-4 plastic resin, and 1 to 1.5-micron thick sections were analyzed using a quantitative computer image analysis system attached to the microscope. Retinal layer thickness (retinal pigment epithelium, RPE; outer nuclear layer thickness, ONL; inner nuclear layer thickness, INL; and length of photoreceptor inner and outer segments, IS+OS) was measured.

Assessment of DNA Changes. Albino rats were euthanized by CO₂ inhalation and individual retinas were frozen in separate tubes. Each retina had been sonicated in 0.8 ml (2.0 M NaCl, 50 mM NaPO₄, pH 7.4, 2 mM EDTA) to yield a uniform homogenate, and stored frozen. Aliquots (0.1 ml) of each sample were diluted 10-fold with 2.0 M NaCl, 50 mM NaPO₄, pH 7.4, 2 mM EDTA containing 1.1 µg/ml bisbenzimidazole (Hoechst 33258). A standard curve was constructed using calf thymus DNA from 0 to 25 µg/ml in the same buffer. Triplicate 0.2 ml aliquots of each retina sample and standard were pipetted into a 96 well plate for fluorescence measurements in the Cytofluor II. The excitation wavelength was 360 nm, and the emission wavelength was 460 nm.

Subjects and Dosing. Male Sprague Dawley rats were randomly assigned to drug and vehicle experimental groups. Control rats were housed in their home cage under normal cyclic light exposure. All rats were dosed 48, 24 and 0 hours prior to a 6-hour blue-light exposure. Dosing was as follows:

1.) **8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin):** Rats receiving either vehicle (N=10) or 8-OH-DPAT (0.5 mg/kg [N=5] or 1.0 mg/kg [N=10]) were given three subcutaneous (SC) injections prior to light exposure. Five rats were used as controls. Retinal protection was assessed by analyzing the ERG response and measuring changes in retinal morphology.

2.) **Buspirone:** For DNA quantitation, six rats per treatment group were dosed IP with vehicle or buspirone (0.5 and 1 mg/kg) prior to light exposure. Retinas from seven normal rats were used as controls. To evaluate changes in retinal morphology, rats were dosed (IP) with either vehicle (N=8) or buspirone (1.0 mg/kg [N=9]). Six rats were used as controls. Retinal protection was assessed by quantitating changes in retinal DNA and measuring changes in retinal morphology.

3.) **SR-57746A**: Rats were dosed (IP) with vehicle (N=15) or SR-57746A (0.5 mg/kg [N=5] or 1 mg/kg [N=15]). Eleven rats were used as controls. The ERG was analyzed after a 5-day recovery period to assess retinal protection.

5 **8-OH-DPAT Evaluation Results.** Blue-light exposure for 6 hours resulted in a significant diminution of the ERG response amplitude (ANOVA, $p < 0.001$; Bonferroni t-test, $p < 0.05$) compared to normals when measured after a 5-day recovery period (Figures 1A and B). Blue-light exposure resulted in a 75% reduction in the maximum a- and b-wave amplitudes in vehicle dosed rats compared to controls.
10 In addition, threshold responses were lower and evoked at brighter flash intensities.

Rats dosed with 8-OH-DPAT showed dose-dependent protection of outer and inner retina function against this photo-oxidative induced retinopathy (Figures 1A and B). Maximum a- and b-wave response amplitudes in 8-OH-DPAT (0.5 mg/kg) dosed
15 rats were not different than vehicle dosed rats and were approximately 27% of control amplitudes. However, maximum a- and b-wave response amplitudes from 8-OH-DPAT (1.0 mg/kg) dosed rats were approximately 53% and 61% of normal, respectively, and significantly higher than responses measured in vehicle dosed rats (Figures 1A and 1B).

20 Consistent with these ERG changes, morphometric analysis of these retinas after a 3-week recovery period demonstrated a significant (ANOVA, $p < 0.01$) loss of photoreceptor cells, shortening of photoreceptor inner + outer segment length, and flattening of the RPE in vehicle dosed animals. No significant changes in the
25 thickness of the INL were detected. ONL thickness was reduced 73%, inner + outer segment length was reduced 82%, and RPE thickness was reduced 59% compared to controls (Figure 2). Lesions observed in rats dosed with 8-OH-DPAT (0.5 mg/kg) were not significantly different than lesions measured in vehicle dosed rats. While ERGs were reduced approximately 63%, the ONL thickness was reduced by 53%,
30 photoreceptor segment length was reduced 60%, and the RPE thickness was reduced 34%. However, photic lesions observed in rats dosed with 8-OH-DPAT (1.0 mg/kg) were significantly different from vehicle dosed rats. While ERG response amplitudes were greater than 50% of normal, the ONL thickness was 2.4 fold thicker, photoreceptor segment length was 2.9 times longer, and RPE thickness was 1.9 times
35 thicker compared to vehicle dosed rats.

Buspiron Evaluation Results. As seen in Figure 3A, vehicle dosed retinal DNA levels were significantly reduced (ANOVA, $p=0.017$) about 30% from control levels.

No significant differences were measured between groups dosed with vehicle or 0.1 mg/kg buspirone. Retinal protection was measured in rats dosed with buspirone (1 mg/kg). Retinal DNA levels were significantly higher than measured in vehicle dosed rats, but not significantly different than controls.

Blue-light exposure for 6 hours resulted in a significant reduction in photoreceptor number (ANOVA, $p < 0.05$). Morphometric analysis of these retinas after a 4-week recovery period demonstrated a 54% thinning of the outer nuclear layer in vehicle dosed rats compared to controls (Figure 3B). However, no significant difference in ONL thickness was measured between normal and buspirone treated rats. In rats dosed with buspirone (1 mg/kg) the ONL thickness was 28.3 μ compared to 30.4 μ in normal rats.

SR-57746A Evaluation Results. Significant protection of retinal function was measured in light-exposed rats dosed with SR-57746A (0.5 and 1.0 mg/kg). Maximum a- and b-wave response amplitudes were reduced by 50% in vehicle dosed rats compared to controls (Figures 4A and B). Maximum responses were 82% of controls in rats dosed with SR-57746A (0.5 mg/kg) and 70% of normal in rats dosed with 1 mg/kg.

Conclusion. These 5-HT_{1A} agonists (8-OH-DPAT, buspirone, and SR-57746A) demonstrated good potency and efficacy in this oxidative model of retinal degenerative disease. Functional and structural protection were achieved in rats dosed on three consecutive days with a dose as low as 1 mg/kg.

We Claim:

1. The use of a pharmaceutically effective amount of a compound with 5-HT_{1A} agonist activity in method of making a medicament for use in a method for
5 treating disorders of the outer retina.

2. The use of Claim 1, wherein the compound is selected from the group consisting of: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-
10 127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, E-5842, SUN-N4057, Org-13011, Org-12966 and 8-OH-DPAT.

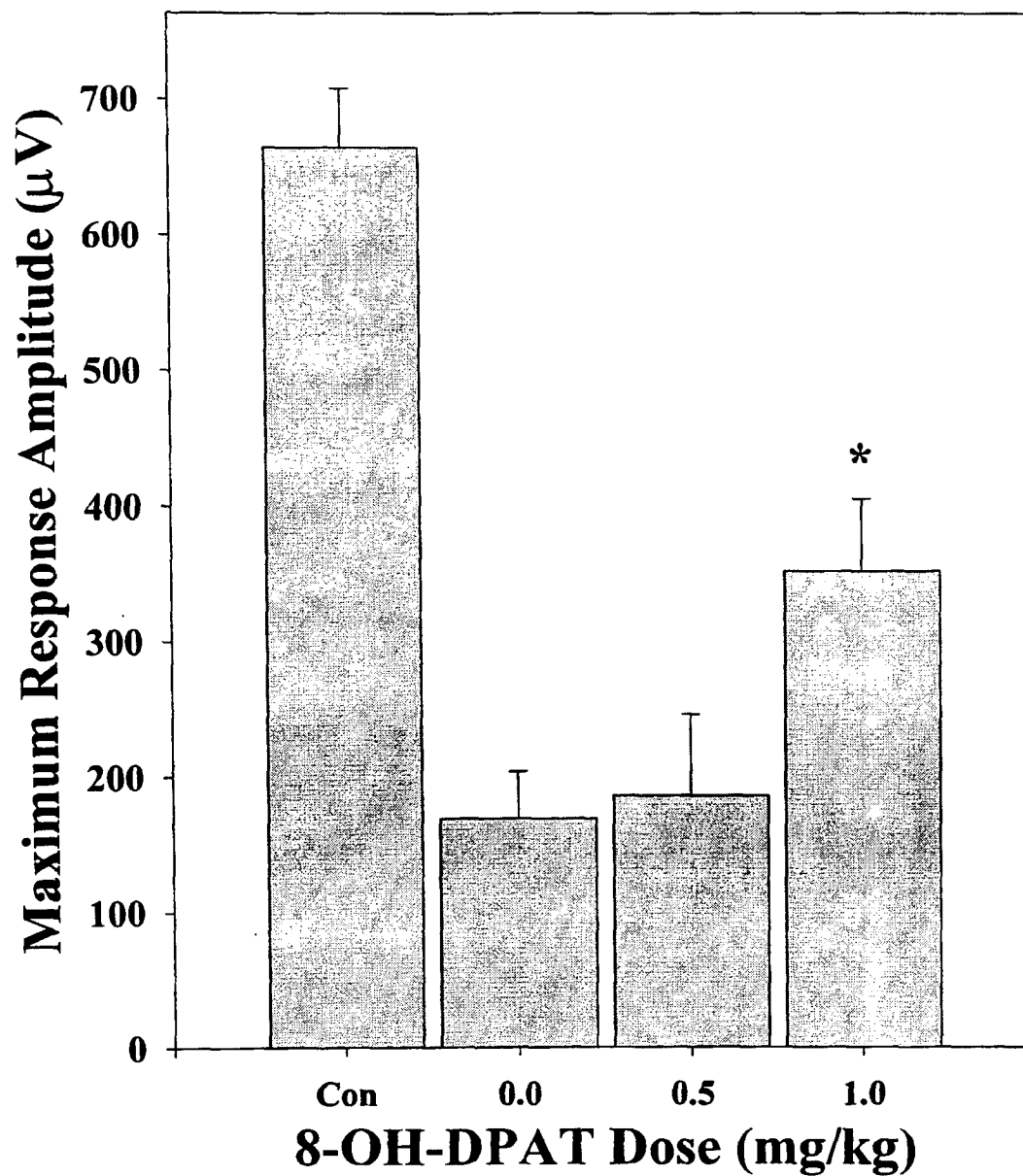
3. The use of Claim 1 wherein the disorder is selected from the group
15 consisting of: AMD; RP and other forms of heredodegenerative retinal disease; retinal detachment and tears; macular pucker; ischemia affecting the outer retina; diabetic retinopathy; damage associated with laser therapy (grid, focal, and panretinal) including photodynamic therapy (PDT); trauma; surgical (retinal translocation, subretinal surgery, or vitrectomy) or light-induced iatrogenic retinopathy; and
20 preservation of retinal transplants.

4. The use of Claim 3 wherein the disorder is AMD.

5. The use of Claim 3 wherein the compound is selected from the group consisting of: tandospirone, urapidil, ziprasidone, repinotan hydrochloride, xaliproden hydrochloride (SR-57746A), buspirone, flesinoxan, EMD-68843, DU-
25 127090, gepirone, alnespirone, PNU-95666, AP-521, flibanserin, MKC-242, lesopitron, sarizotan hydrochloride, E-5842, SUN-N4057, Org-13011, Org-12966 and 8-OH-DPAT.

6. The use of Claim 5 wherein the disorder is selected from the group consisting of AMD, RP, and diabetic retinopathy.

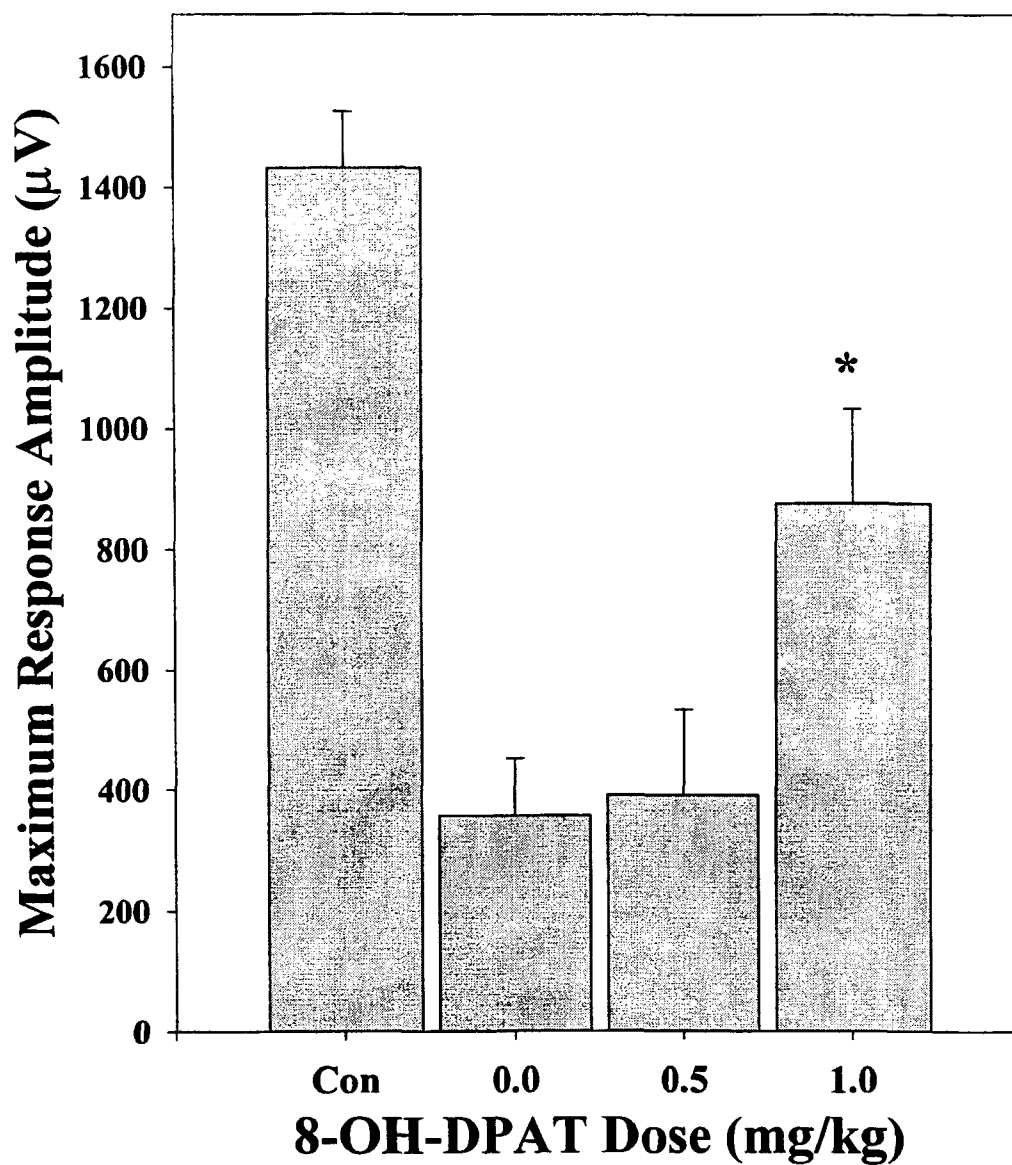
FIGURE 1A

A-wave

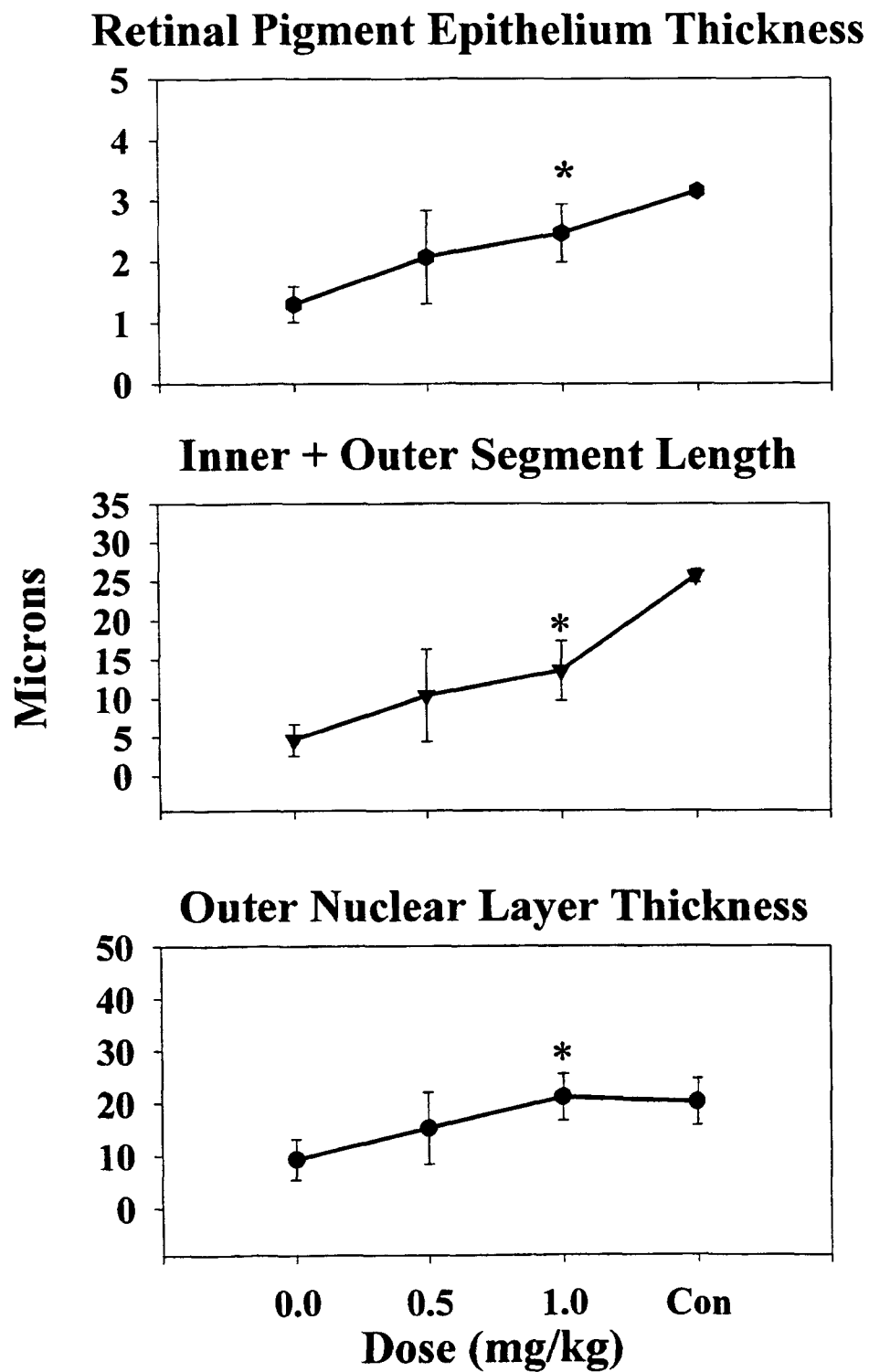
* Significantly higher responses compared to vehicles ($p < 0.05$).

FIGURE 1B

B-wave

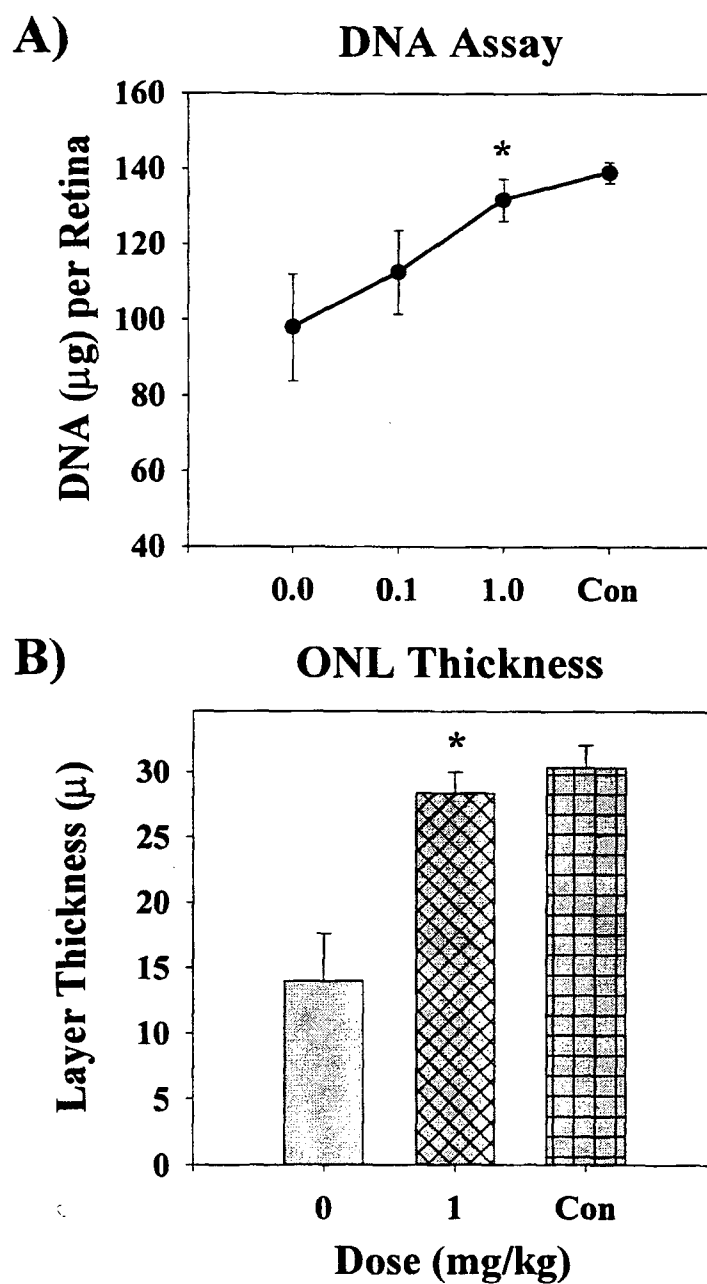


* Significantly higher responses compared to vehicles ($p < 0.05$).

FIGURE 2

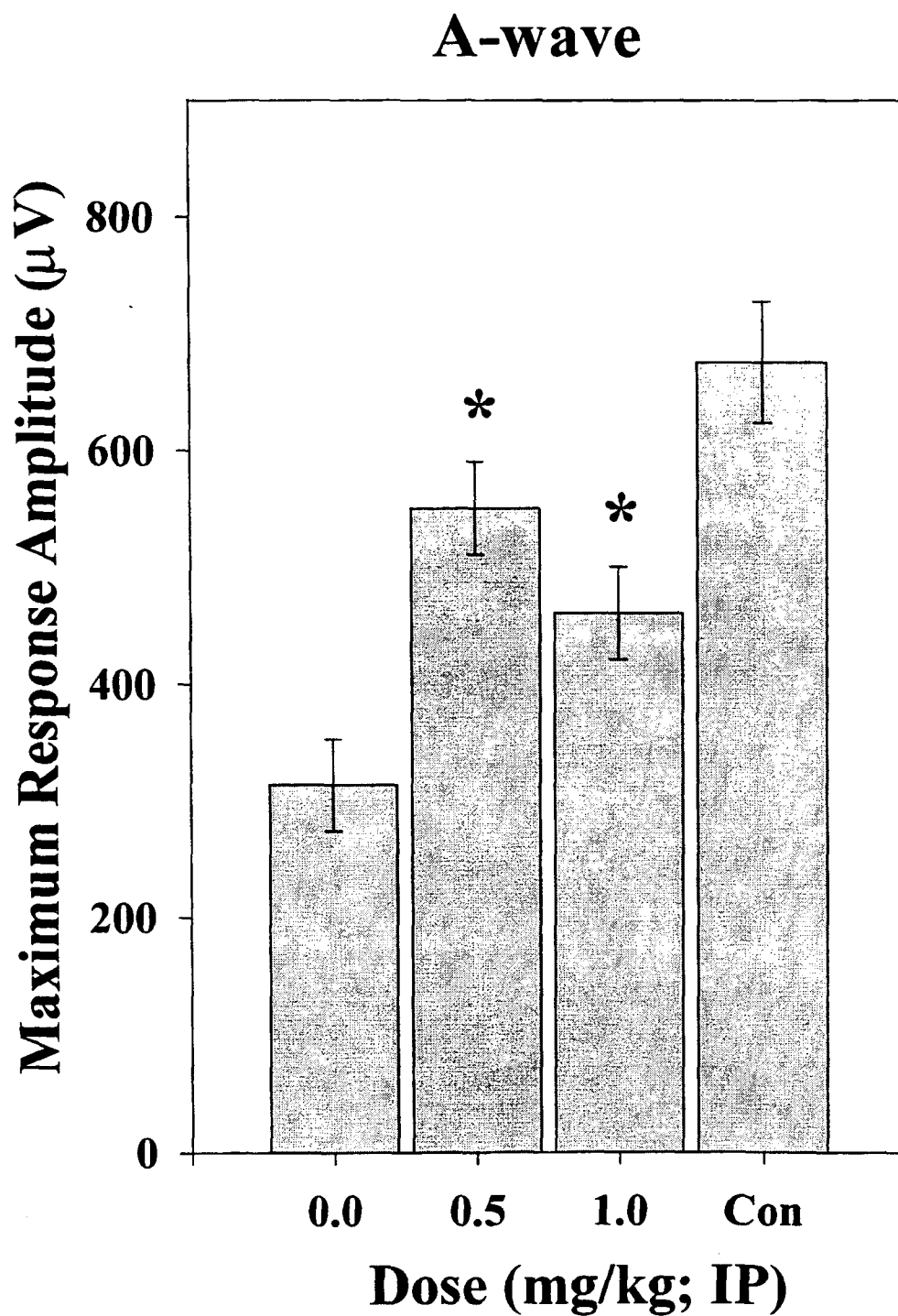
* Significantly less damaged than vehicle ($p < 0.05$).

FIGURE 3



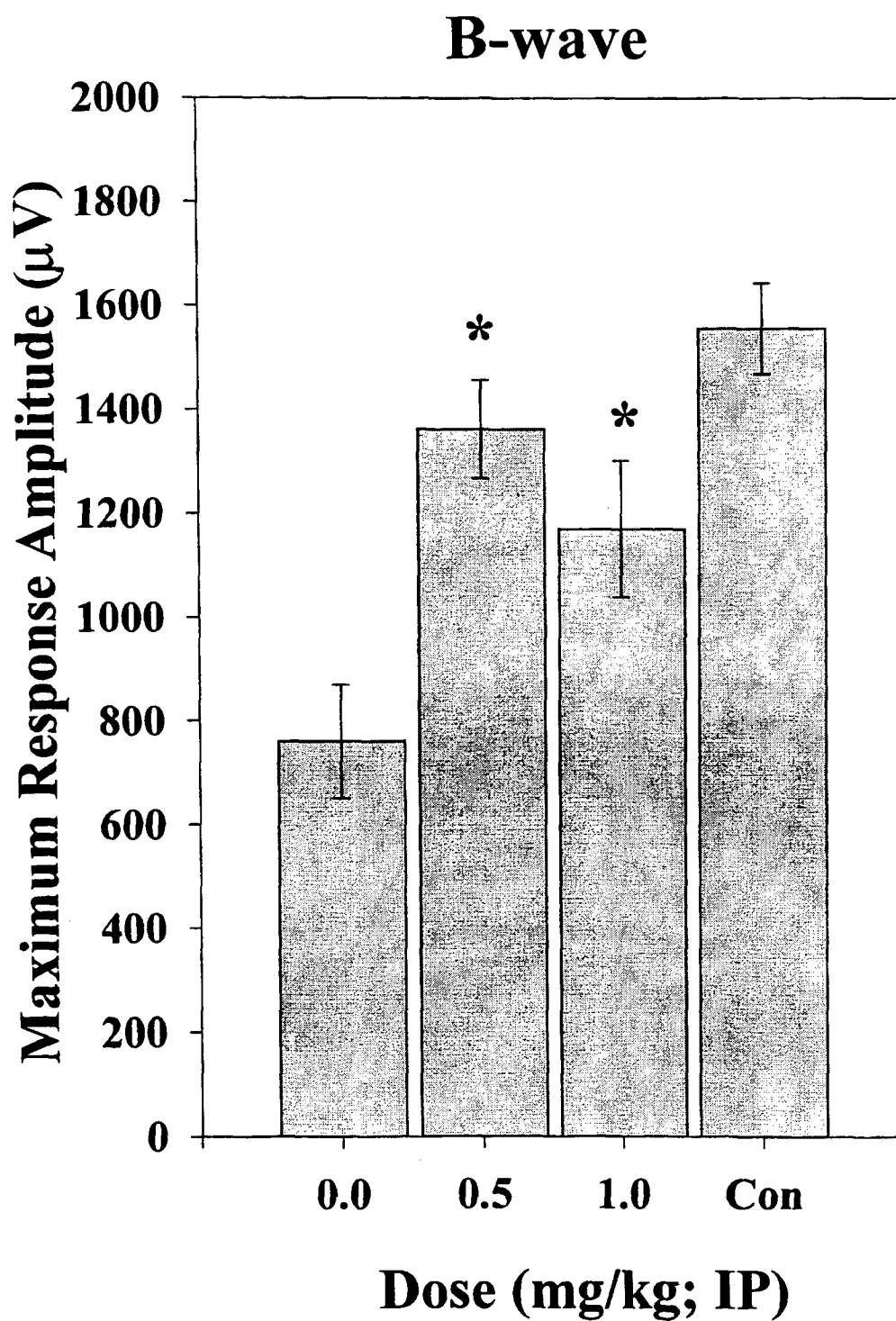
* Significantly better than vehicle ($p < 0.05$) and not different than control.

FIGURE 4A



* Significantly better response compared to vehicle ($p < 0.05$).

FIGURE 4B



* Significantly better response compared to vehicle ($p < 0.05$).