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Rimonabant, a selective cannabinoid$_1$ receptor antagonist, protects against light-induced retinal degeneration *in vitro* and *in vivo*

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ABSTRACT

The endocannabinoid system is involved in some neurodegenerative diseases such as Alzheimer's disease. An endogenous constellation of proteins related to cannabinoid$_1$ receptor signaling, including free fatty acids, diacylglycerol lipase, and N-acylethanolamine-hydrolyzing acid amidase, are localized in the murine retina. Moreover, the expression levels of endogenous agonists of cannabinoid receptors are changed in the vitreous fluid. However, the role of the endocannabinoid system in the retina, particularly in the light-induced photoreceptor degeneration, remains unknown. Therefore, we investigated involvement of the cannabinoid$_1$ receptor in light-induced retinal degeneration using *in vitro* and *in vivo* models. To evaluate the effect of cannabinoid$_1$ receptors in light irradiation-induced cell death, the mouse retinal cone-cell line (661 W) was treated with a cannabinoid$_1$ receptor antagonist, rimonabant. Time-dependent changes of expression and localization of retinal cannabinoid$_1$ receptors were measured using Western blot and immunostaining. Retinal damage was induced in mice by exposure to light, followed by intravitreal injection of rimonabant. Electroretinograms and histologic analyses were performed. Rimonabant suppressed light-induced photoreceptor cell death. Cannabinoid$_1$ receptor expression was upregulated by light exposure. Treatment with rimonabant improved both a- and b-wave amplitudes and the thickness of the outer nuclear layer. These results suggest that the cannabinoid$_1$ receptor is involved in light-induced retinal degeneration and it may represent a therapeutic target in the light-induced photoreceptor degeneration related diseases.

1. Introduction

Excessive light exposure is known to induce retinal damage, particularly of photoreceptors (Wenzel et al., 2005). Photoreceptor loss is the primary cause of blindness such as that seen in age-related macular degeneration (AMD) (Hunter et al., 2012) and retinitis pigmentosa. In a previous report, exposure to light appeared to affect the incidence of AMD (Wenzel et al., 2005). However, there are few effective therapies for these diseases. Therefore, it is important to investigate the mechanism of light-induced photoreceptor degeneration and therapeutic methods, including photoreceptor-protective agents.

Recently, it was revealed that certain G-protein coupled receptors, such as adrenergic and serotonin receptors, are related to photoreceptor cell damage *via* G-protein coupled receptor signaling (Chen et al., 2013). However, the role of many G-protein coupled receptors in the retina remains controversial. Cannabinoid receptors are classified as G-protein coupled receptors and have a wide variety of functions. Cannabinoid$_1$ and cannabinoid$_2$ receptors are the most active endocannabinoid receptors. Cannabinoid$_1$ receptors are known to be localized in some nerve cells, including the brain (Robinson et al., 2016) and spinal cord (Paniagua-Torija et al., 2015). They have important functions, such as regulating the release of neurotransmitters from nerve terminals (Lee et al., 2015). Therefore, changes downstream of cannabinoid$_1$ receptor signaling have long been assumed to play a pivotal role in several neurodegenerative and neuroinflammatory disorders, such as Alzheimer disease (Bedse et al., 2015, 2014), Parkinson disease (Cerri et al., 2014), Huntington disease (Naydenov et al., 2014), amyotrophic lateral sclerosis (Bilsland et al., 2006), and multiple sclerosis (Mori et al., 2014) In addition, Alzheimer disease is associated with AMD (Fisichella et al., 2016; Keenan et al., 2014). In a previous report, a constellation of proteins

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related to cannabinoid$_1$ receptor signaling, including free fatty acids, diacylglycerol lipase, and N-acylethanolamine-hydrolyzing acid amidase, were found to be localized in the retinas of rats (Cecyre et al., 2014) and mice (Hu et al., 2010). The levels of two endogenous agonists of the cannabinoid$_1$ receptor, anandamide (arachidonoylethanolamide) and 2-arachidonoyl-glycerol, are upregulated in the vitreous fluid of patients with AMD compared with that of healthy individuals (Matias et al., 2006). These findings suggest that the cannabinoid$_1$ receptor is related in some way to retinal function and the pathophysiology of AMD, even though these functions have not yet been studied in detail. Therefore, we designed this study to investigate the role of the cannabinoid$_1$ receptor on light-induced retinal damage in mice using the selective cannabinoid$_1$ receptor antagonist rimonabant (SR141716).

2. Material and methods

2.1. Cell culture

The mouse retinal cone-cell line 661 W, a transformed mouse cone-cell line derived from murine retinal tumors, was provided by Dr. Muayyad R. Al-Ubaidi (University of Houston, Houston, TX, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The cells were passaged by trypsinization every three to four days.

2.2. Exposure of murine retinal cone cells to white light

The 661 W cells were seeded at 3×10^3 cells per well in 96-well plates and then incubated for 24 h. The entire medium was then replaced with fresh medium containing 1% fetal bovine serum. Rimonabant (Cayman Chemical, Ann Arbor, MI, USA) at 1–100 nM and trolox (Wako, Osaka, Japan) at 100 nM were added and, 1 h following treatment, the cells were exposed to 2500 lx of white fluorescent light (C-FPS115D; Nikon, Tokyo, Japan) for 24 h at 37 °C. The luminance was measured using a light meter, LM-332 (As One, Osaka, Japan). Nuclear staining assays were performed after an additional 24 h of incubation.

2.3. Nuclear staining assays

At the end of the culture period, Hoechst 33342 (λ_{ex}=360 nm, λ_{em} > 490 nm) and propidium iodide (Thermo Fisher Scientific, Waltham, MA, USA) (λ_{ex}=535 nm, λ_{em} > 617 nm) were added to the culture medium for 15 min at final concentrations of 8.1 μM and 1.5 μM, respectively. Hoechst 33342 freely enters living cells and stains the nuclei of viable cells, as well as those that have suffered apoptosis or necrosis. Propidium iodide is a membrane-impermeable dye that is generally excluded from viable cells. Images were collected using an Olympus IX70 inverted epifluorescence microscope (Olympus, Tokyo, Japan). We counted the total number of cells and calculated the percentage of propidium iodide-positive cells as a measure of dead cells.

2.4. Immunostaining

The cells were seeded on a chamber slide (Nunc, Rochester, NY, USA). After 24 h, the cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde. Cells were incubated with anti-cannabinoid$_1$ receptor antibody (Cell Signaling Technology, Danvers, MA, USA) followed by Alexa Fluor 488-conjugated secondary antibodies (Thermo Fisher Scientific). Nuclei were stained with Hoechst 33342. Stained cells were examined using a confocal laser scanning microscope (FV10i-DOC; Olympus).

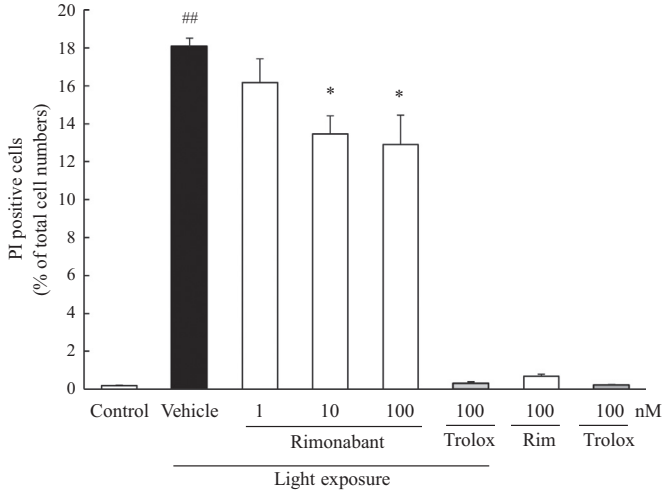

Fig. 1. Effect of cannabinoid$_1$ receptor on light-induced photoreceptor cell death. Rimonabant or trolox was added 1 h before light exposure. The number of cells exhibiting propidium iodide fluorescence was counted and expressed as the percentage of propidium iodide-positive cells to Hoechst 33342 positive cells. Data are expressed as mean ± S.E.M. (n =6). $^{\#\#}$ $P<0.01$ versus control (t-test); * $P<0.05$, ** $P<0.01$ *vs.* vehicle (Dunnett's test).

2.5. Animals

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved and monitored by the Institutional Animal Care and Use Committee of Gifu Pharmaceutical University. Male albino ddY mice (Japan SLC, Inc., Hamamatsu, Japan), aged 9–10 weeks, were used in this study. They were kept under controlled lighting conditions (12-h light/dark cycle).

2.6. Exposure to light

After dark adaptation for 24 h, the pupils of the mice were dilated with 1% cyclopentolate hydrochloride eye drops (Santen, Osaka, Japan) 30 min before exposure to light. Unanesthetized mice were exposed to 8000 lx of white fluorescent light (Toshiba, Tokyo, Japan) for 3 h in cages with a reflective interior. The temperature during exposure to light was maintained at 25 ± 1.5 °C. After exposure to light, all mice were returned to darkness for 24 h and then placed in the normal light/dark cycle.

2.7. Treatment with rimonabant

Rimonabant was suspended in PBS containing 0.1% dimethyl sulfoxide and, in mice anesthetized with isoflurane, injected into the vitreous space (final doses were 1 and 10 μM/eye) by puncturing the eye at the corneal-scleral junction using a syringe equipped with a 33-gauge needle. This was done at 1 h before light exposure. The vehicle group was injected with PBS containing 0.5% dimethyl sulfoxide. The injection volume was 2 μL in all cases.

2.8. Electroretinogram

Electroretinograms (ERG) were recorded 5 days after light exposure (Mayo, Aichi, Japan). Mice were maintained in a completely dark room for 24 h. They were anesthetized with a mixture of ketamine (120 mg/kg i.p.; Daiichi-Sankyo, Tokyo, Japan) and xylazine (6 mg/kg i.p.; Bayer Health Care, Tokyo, Japan). Pupils were dilated with 1% tropicamide and 2.5% phenylephrine (Santen, Osaka, Japan). Flash ERG was recorded in the left eyes of dark-adapted mice by placing a gold ring electrode (Mayo) in contact with the cornea and a reference