

Involvement of Cannabinoid Receptors in the Intraocular Pressure-Lowering Effects of WIN55212-2¹

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ABSTRACT

It is known that marijuana smoking and administration of natural cannabinoids reduce intraocular pressure. However, it has not been established whether the intraocular pressure-lowering effects of cannabinoids are mediated by cannabinoid receptors. Aminoalkylindoles are a new class of cannabimimetics with structures entirely different from those of natural cannabinoids. WIN55212-2, a prototypic aminoalkylindole, has been shown to bind cannabinoid receptors and to exhibit cannabinoid-like activities. The objective of this study was to determine whether aminoalkylindoles lower intraocular pressure and whether the effects of aminoalkylindoles are mediated by ocular cannabinoid receptors. The intraocular pressure of New Zealand White rabbits was measured with the use of applanation pneumatonography. After the measurement of baseline intraocular pressure, drugs were applied topically and the intraocular pressure was monitored. The topical application of

WIN55212-2 significantly reduced intraocular pressure in the treated eyes. The intraocular pressure-lowering effects of WIN55212-2 were time and dose dependent, and the maximal reduction was 4.7 ± 0.5 mm Hg at a dose of 100 μ g. In contrast to treated eyes, the intraocular pressure on the contralateral eyes was not significantly affected. The topical application of WIN55212-3, the enantiomer of WIN55212-2, had no effect on intraocular pressure. Furthermore, the intraocular pressure-lowering effects of WIN55212-2 were significantly reduced by topically administered SR141716A, a selective antagonist for the CB1 cannabinoid receptor. The dose-response curve of WIN55212-2 is shifted parallel to the right by SR141716A. These data demonstrate that like natural cannabinoids, WIN55212-2 also reduces intraocular pressure, and the effects of WIN55212-2 are mediated at least in part by the CB1 cannabinoid receptors in the eye.

Marijuana (*Cannabis sativa*) is one of the oldest and most widely abused drugs. The primary psychoactive active constituent of marijuana is Δ^9 -tetrahydrocannabinol (Δ^9 -THC; Gaoni and Mechoulam, 1964). In addition to psychotropic activity, Δ^9 -THC and other cannabinoids produce a variety of effects with therapeutic potentials, such as analgesia, anti-nausea, immunosuppression, and intraocular pressure (IOP) decrease (Hollister, 1986). To date, cannabinoids have been found to act through G protein-coupled receptors (Devane et al., 1988). Several cDNAs and genes encoding cannabinoid receptors have been cloned, including CB1 and CB2 (Matsuda et al., 1990; Munro et al., 1993). The endogenous cannabinoid ligand anandamide has been isolated from the brain (Devane et al., 1992).

Marijuana smoking was first reported to reduce IOP in 1971 (Hepler and Frank, 1971). After this initial observation, many studies have been conducted on human subjects and animal models that confirmed the IOP-lowering properties of

marijuana, Δ^9 -THC, and classic cannabinoid derivatives (Flom et al., 1975; Purnell and Gregg, 1975; Green, 1984; Colasanti, 1986). Recently, anandamide, an endogenous cannabinoid agonist, also was found to lower IOP after topical administration to the rabbit eye (Pate et al., 1995). It has been suggested that compounds from the classic cannabinoid family may lower IOP by reducing aqueous humor formation and by enhancing aqueous humor outflow in the anterior chamber of the eye (Colasanti, 1986). However, the precise mechanisms for the IOP-lowering effects of cannabinoids have not been elucidated. It is not clear whether cannabinoid receptors are involved in the IOP-lowering effects of cannabinoids.

Aminoalkylindoles are a class of compounds with chemical structures entirely different from those of natural cannabinoids (Compton et al., 1992; D'Ambra et al., 1992). Aminoalkylindoles bind to cannabinoid receptors and exhibit cannabinoid-like activity in vitro and in vivo. In this study, the IOP-regulating effects of a prototypical aminoalkylindole, WIN55212-2, and its inactive enantiomer, WIN55212-3, were investigated. In addition, SR141716A, a selective antagonist for the CB1 cannabinoid receptor (Rinaldi-Carmona et al.,

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ABBREVIATIONS: Δ^9 -THC, Δ^9 -tetrahydrocannabinol; IOP, intraocular pressure.

1994; Bouaboula et al., 1997), was used to examine whether the CB1 cannabinoid receptors play a role in the ocular effects of WIN55212-2.

Materials and Methods

Experimental Animals. New Zealand White rabbits (2.5–3.5 kg) of either sex were used. Rabbits were housed with a 12-h light/dark cycle and maintained on rabbit chow and water ad libitum. National Institutes of Health and Association for Research in Vision and Ophthalmology guidelines for the use and care of animals were followed in this study.

Drug Preparation and Administration. WIN55212-2 and WIN55212-3 were purchased from Research Biochemicals, Inc. (Natick, MA). SR141716A was obtained from the Research Biochemicals through the National Institute of Mental Health drug supply program.

Because cannabinoid ligands are not very water soluble, a vehicle of 45% 2-hydroxypropyl- β -cyclodextrin (Research Biochemicals) was used. This vehicle has been successfully used to deliver anandamide, a putative endogenous cannabinoid agonist, to rabbit eyes and has been found to stabilize anandamide in solutions (Jarho et al., 1996). Compared with mineral oil, another vehicle that is used for the topical application of cannabinoids, 2-hydroxypropyl- β -cyclodextrin is not irritating or toxic to the eye. Drugs and vehicle were administered topically in a 50- μ l volume. Before the measurement of IOP, 10 μ l of 0.05% tetracaine eyedrops (Optics Laboratories, Fairton, NJ) was applied topically for local anesthesia. All measurements of IOP were made under local anesthesia, and tetracaine did not significantly affect IOP.

Measurement of IOP. IOP was measured with an Alcon (Fort Worth, TX) applanation pneumatonograph that has been calibrated manometrically for rabbit eyes. Baseline IOP was measured at 0.5 and 0 h before drug administration, and IOP values from the two readings were averaged to provide a zero time value for the animal. After the unilateral topical administration of cannabinoid agonists or vehicle, the IOP of both eyes was monitored 0.5, 1, 2, 3, 4, and 5 h after drug administration. Experiments were performed in a masked design (i.e., the individual performing the IOP measurements had no prior knowledge of the drug being administered). For the antagonism experiments, the antagonist was administered at 0.5 h before the application of the agonists. The effects of the antagonist alone on IOP were also determined.

Data Analysis and Statistics. Six rabbits were used for each group of treatment. Each rabbit was used only once. The data presented in the figures represent mean $\pm$ S.E. values. The data were analyzed using Prism (GraphPAD Software, San Diego, CA) and plotted as change in IOP (mm Hg) versus time (h). Student's *t* tests or one-way ANOVA with Bonferroni's post hoc tests were used to compare the data points of different treatment groups. The level of significance was chosen as $P < .05$.

Results

IOP-Lowering Effects of WIN55212-2. Figure 1 shows the time course and dose-response relationships for the effects of WIN55212-2 on IOP. At a dose of 100 μ g, WIN55212-2 produced a significant reduction of IOP in the drug-treated eyes at 1, 2, and 3 h after unilateral topical application. The IOP-lowering effects of WIN55212-2 peaked between 1 and 2 h after WIN55212-2 administration, and IOP returned to control level at 4 h after WIN55212-2 administration. The IOP-lowering effects of WIN55212-2 were dose dependent. The IOP-lowering effects produced by 20 μ g of WIN55212-2 were less than those produced by 100 μ g of WIN55212-2, and 4 μ g of WIN55212-2 did not produce significant IOP-lowering effects. The duration

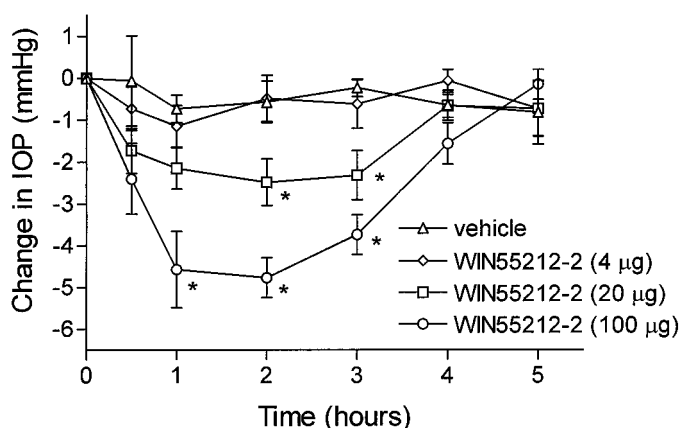


Fig. 1. Time course and dose-response relationship of the IOP changes (treated eye) induced by topically administered WIN55212-2. Values represent the mean $\pm$ S.E. of six animals. *, significant differences ($P < .05$) between the drug-treated and the vehicle-treated group.

of action for 20 μ g of WIN55212-2 was similar to that of 100 μ g. The maximal IOP reduction by 100 μ g of WIN55212-2 was 4.7 ± 0.5 mm Hg. With 2-hydroxypropyl- β -cyclodextrin as a vehicle, 100 to 200 μ g of WIN55212-2 was the highest achievable dose because at higher concentrations, WIN55212-2 became insoluble.

Figure 2 demonstrates the change in IOP in the eye contralateral to the eye receiving topical administration of 100 μ g of WIN55212-2. Compared with contralateral vehicle administration, there was no significant reduction of IOP at any time after contralateral WIN55212-2 administration.

Figure 3 illustrates the stereoselectivity for the IOP-lowering effects of WIN55212-2. The topical application of 100 μ g of WIN55212-3, the enantiomer of WIN55212-2, did not lower IOP.

Antagonism of IOP-Lowering Effects of WIN55212-2. Figure 4 shows the effects on IOP of the selective CB1 receptor antagonist SR141716A alone. At a dose of 25 μ g, SR141716A produced a significant increase in IOP at 0.5 and 1 h after topical administration. This effect of SR141716A had a short duration; the IOP returned to the control level at 2 h after administration. Due to limited solubility, this dose of SR141716A is the highest achievable dose in the vehicle

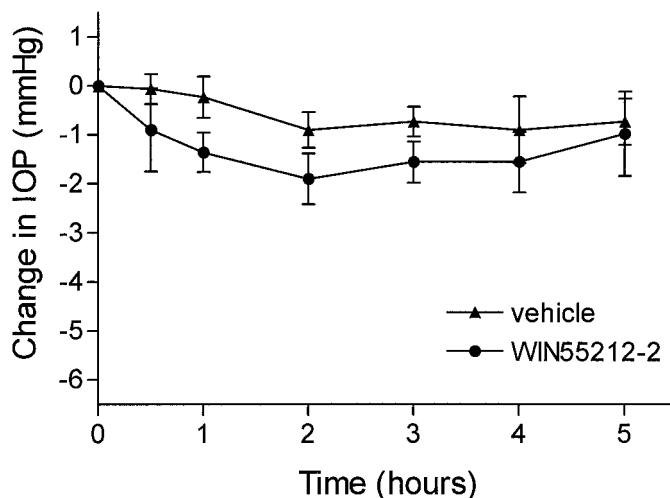


Fig. 2. IOP changes in the eye contralateral to the eye with topical administration of WIN55212-2. A dose of 100 μ g of WIN55212-2 was used. Values represent the mean $\pm$ S.E. of six animals.

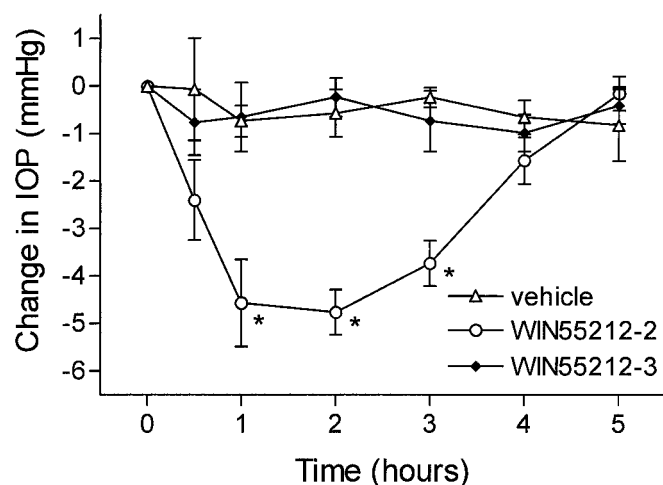


Fig. 3. Comparison of the IOP changes (treated eye) induced by topically administered WIN55212-2 and WIN55212-3. A dose of 100 μ g of WIN55212-2 or 100 μ g of WIN55212-3 was used. Values represent the mean $\pm$ S.E. of six animals. *, significant differences ($P < .05$) between the drug-treated and the vehicle-treated group.

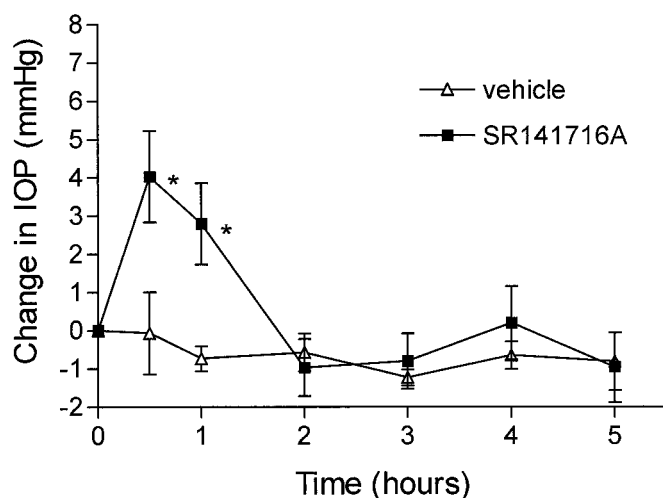


Fig. 4. IOP changes (treated eye) induced by topically administered SR141716A. A dose of 25 μ g of SR141716A was used. Values represent the mean $\pm$ S.E. of six animals. *, significant differences ($P < .05$) between the drug-treated and the vehicle-treated group.

2-hydroxypropyl- β -cyclodextrin. In contrast to the treated eyes, SR141716A had no significant effect on IOP in the contralateral eyes (data not shown).

Figure 5 demonstrates the antagonistic effects of SR141716A on the IOP-lowering effects of WIN55212-2. The application of 25 μ g of SR141716A at 0.5 h before WIN55212-2 administration significantly attenuated the IOP-lowering effect of 100 μ g of WIN55212-2 at 1, 2, and 3 h after WIN55212-2 administration.

Figure 6 shows the dose-response relationship of the antagonism produced by SR141716A on the IOP-lowering effects of WIN55212-2. The dose-response curve of WIN55212-2 was shifted parallel to the right by SR141716A.

Discussion

Cannabinoid agonists can be classified into at least four chemical classes: classic cannabinoids (Gaoni and Mechoulam, 1964; Mechoulam et al., 1988), bicyclic cannabinoids (Johnson

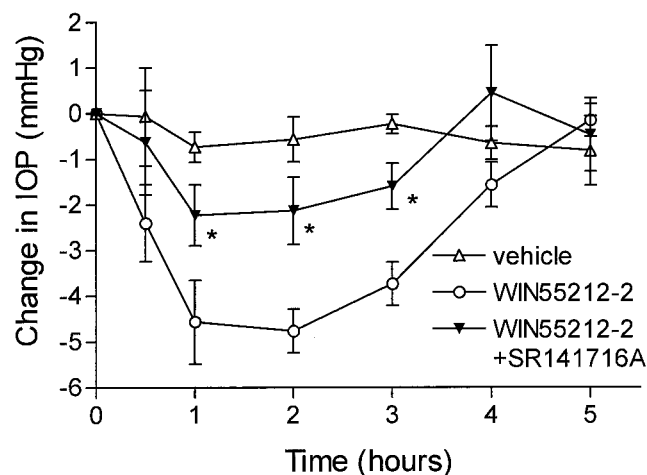


Fig. 5. Antagonism of the IOP-lowering effects of WIN55212-2 by topically administered SR141716A. A dose of 25 μ g of SR141716A was administered at 0.5 h before the administration of 100 μ g of WIN55212-2. Values represent the mean $\pm$ S.E. of six animals. *, significant differences ($P < .05$) between the drug-treated groups (WIN55212-2 + SR141716A and the WIN55212-2 alone).

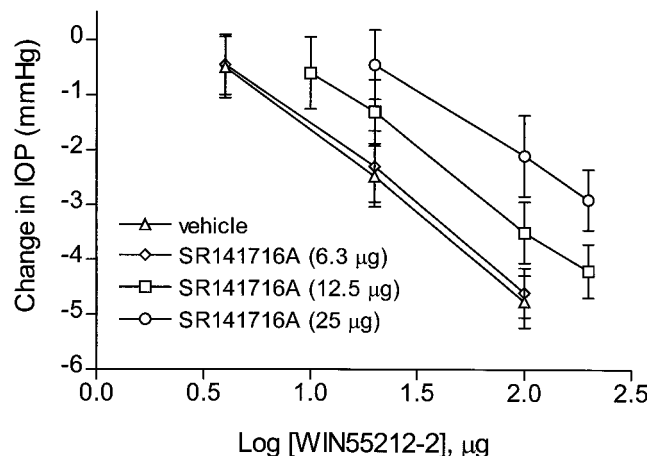


Fig. 6. Dose-response relationship for the antagonism of the IOP-lowering effects of WIN55212-2 by SR141716A. Doses of 6.3, 12.5, and 25 μ g of SR141716A was administered at 0.5 h before the administration of different doses of WIN55212-2. The IOP was measured at 2 h after WIN55212-2 administration. Values represent the mean $\pm$ S.E. of six animals.

and Melvin, 1986; Melvin et al., 1993), fatty acid amides and esters (Devane et al., 1992; Mechoulam et al., 1995), and aminoalkylindoles (Compton et al., 1992; D'Ambra et al., 1992). The IOP-lowering effects have been reported for classic cannabinoids (Purnell and Gregg, 1975; Green, 1984; Colasanti, 1986), bicyclic cannabinoids (Pate et al., 1998), and fatty acid amides (Pate et al., 1995, 1998). However, it is not clear whether aminoalkylindoles can produce IOP-lowering effects. Recently, Hodges et al. (1997) reported that the i.v. administration of WIN55212-2 has no significant effect on IOP. In the current study, a reduction in IOP was observed after the topical application of WIN55212-2. Thus, our data are inconsistent with those of Hodges et al. (1997). At present, the reasons behind this inconsistency are not clear; one possible explanation could be the difference in routes of drug administration. In the current study, we used topical application, whereas Hodges et al. (1997) used i.v. administration. It is possible that through i.v. administration, WIN55212-2 is metabolized before it can reach its ocular target at a sufficient concentration to exert its effects.

In this study, we observed that the IOP-lowering effects of WIN55212-2 were time and dose dependent. Also, we found a difference in potency between the enantiomer pairs of WIN55212-2 and WIN55212-3. In addition, the IOP-lowering effects of WIN55212-2 were attenuated significantly by SR141716A, a highly selective antagonist for the CB1 cannabinoid receptor. Furthermore, the dose-response curve of WIN55212-2 was shifted parallel to the right by SR141716A. Taken together, these data strongly support the hypothesis that the IOP-lowering effects of WIN55212-2 involve the CB1 cannabinoid receptor. Because there is no significant IOP-lowering effect of WIN55212-2 in the eye contralateral to the eye to which the drug was administered, this indicates that the IOP-lowering effects of WIN55212-2 were not results of systemic absorption but rather were mediated by the cannabinoid receptors in the eye.

Presently, there is inconsistency in the literature with regard to whether cannabinoid receptors are involved in the IOP-lowering effects of cannabinoids. It has been demonstrated by Pate et al. (1998) that the IOP-lowering effects of CP-55940, a bicyclic cannabinoid agonist, were blocked by the CB1 antagonist SR141716A. Thus, our data and those of Pate et al. (1998) support the hypothesis that the IOP-lowering effects of cannabinoids involve CB1 cannabinoid receptors in the eye. This hypothesis is further supported by a recent report that CB1 receptor mRNA is expressed in the tissues of anterior chambers of the eye, such as the ciliary body (Porcella et al., 1998). However, there are reports suggesting that cannabinoid receptors are not involved in the IOP-lowering effects of cannabinoids. For example, HU-211, the enantiomer of the classic cannabinoid agonist HU-210, has very weak affinity for the CB1 cannabinoid receptor (Mechoulam et al., 1988); nevertheless, it has potent IOP-lowering effects (Beilin et al., 1993). Even through the reasons for these discrepancies are not clear, it is possible that some of the IOP-lowering effects of cannabinoids are mediated through cannabinoid receptors and that some of the effects are independent of cannabinoid receptors.

In this study, we found that SR141716A by itself produces an increase in IOP. This finding is consistent with that of Pate et al. (1998), who reported an IOP-elevating effect after the i.v. administration of SR141716A. There are at least two possible explanations for SR141716A-induced IOP elevation. First, the increase in IOP caused by SR141716A may be due to its inverse agonist activity at ocular receptors, because SR141716A has been reported to be an inverse agonist at the CB1 cannabinoid receptors (Bouaboula et al., 1997). Second, this may be due to the blockade of the possible tonic regulatory effects of endogenous cannabinoids on IOP. In support of this second possibility, the enzymes for metabolizing endogenous cannabinoids have been localized in ocular tissues (Matsuda et al., 1997).

In summary, this study demonstrates that the topical application of WIN55212-2 lowers IOP and that the IOP-lowering effects of WIN55212-2 are due, at least in part, to its activity on the ocular CB1 cannabinoid receptors. Further studies are needed to elucidate the molecular mechanisms underlying the IOP-lowering effects of cannabinoids through cannabinoid receptor-dependent and possible receptor-independent pathways.

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