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Ocular Activity of Topically Administered Anandamide in the Rabbit

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Abstract: Marijuana and its psychoactive constituents, cannabinoids, reduce intraocular pressure (IOP) in humans and animals. Because anandamide, a recently discovered endogenous ligand of cannabinoid receptors, reportedly shows cannabimimetic pharmacological activity, we examined its effect on the eye. Varying amounts of anandamide, in 50 μL of light mineral oil, were topically applied to a rabbit eye and changes in IOP and ocular symptoms were monitored. Anandamide (50 μg to 1 mg) induced a significant decrease in IOP within 1 hour after administration; maximum reduction occurred at 2 hours; and there was a return to baseline by 7 hours following administration. A noticeable hyperemia of conjunctival blood vessels was also noted 2 hours after administration. Neither mineral oil alone or with palmitylethanolamide (an analogue of anandamide) caused a significant decrease in IOP or conjunctival hyperemia. This study indicates that anandamide does produce cannabimimetic effects in rabbit eyes. **Jpn J Ophthalmol 1997;41:217–220** © 1997 Japanese Ophthalmological Society

Key Words: Anandamide, cannabinoid, intraocular pressure, rabbit.

Introduction

Cannabinoids are psychoactive constituents of marijuana; their psychotropic and pharmacological actions in animals and man are well-documented.[1] The first report on the ophthalmological effects of cannabinoids noted that smoking marijuana lowered intraocular pressure (IOP) in human volunteers.[2] A representative psychoactive cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC), was then shown to reduce IOP in rabbits by 20–25% after intravenous or topical administration.[3,4]

A specific receptor for cannabinoids was recently found in the rat brain,[5] and the cDNA has been cloned both from rat[6] and human[7] tissues. An endogenous ligand of a cannabinoid receptor was then isolated from a porcine brain[8] and identified as arachidonylethanolamide, referred to as anandamide. Similar to Δ^9-THC and other high-affinity ligands of the cannabinoid receptor, anandamide inhibited adenylyl cyclase activity and calcium currents in vitro[9,10] and caused a variety of in vivo effects including hypomotility, hypothermia, antinociception, and catalepsy in mice;[11,12] however, there are few studies of the pharmacological activity of anandamide in the eye.[13,14] We have therefore investigated the ophthalmological effects of topically applied anandamide.

Materials and Methods

Animals

Male albino New Zealand rabbits, 2–3 kg (Japan SLC, Hamamatsu) were maintained on a 12-hour alternating light/dark schedule in an isolated, constant-temperature environment with food and water available ad lib. Animals were given a 2-week rest period between experiments to allow for clearance of the drug. Studies described in this paper are in accord with the Declaration of Helsinki and the Guide for Care and Use of Laboratory Animals, as well as the ARVO statement on the use of animals in ophthalmic and vision research.

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Methods

We tested the effect of light mineral oil, the vehicle used in this study, and confirmed that there was no significant effect on IOP under the experiment conditions (data not shown). With rabbits wrapped safely in cotton blankets, a local anesthetic (0.4% oxybuprocaine) was applied topically and the IOP was measured in room light with an applanation pneumatonograph (Alcon Surgical, Fort Worth, TX, USA), calibrated for use with rabbits. Special care was taken to avoid pressure on the eyeball or surrounding tissues.

Ethanol solutions of anandamide (arachidonylethanolamide) and palmitylethanolamide (Cayman, Ann Arbor, MI, USA) were transferred to 1.5 mL plastic tubes and evaporated on ice in nitrogen gas; the dried product was then dissolved in light mineral oil (Sigma, St. Louis, MO, USA). A fresh solution was prepared for each topical administration. One drop (50 μL) of either the anandamide (500 μg) or palmitylethanolamide (500 μg) solution was applied to one eye of each animal at time 0. For negative control, pure light mineral oil was applied to the same eyes on other days. Nine rabbits were used for repeated experiments with varying conditions. All experiments began at 1300; IOP was measured at time 0 and 0.5, 1, 2, 3, 5, 7, and 16 hours after administration. For dose-response studies, the IOP was measured 2 hours following administration. For statistical analysis, we used the Wilcoxon matched-pairs signed ranks test (Figure 1) or 2-way ANOVA with Bonferroni's multiple comparison to compare averages of the groups (Figure 2). Hyperemia and flare were evaluated with a slit-lamp; pupil size was measured horizontally, by ruler, under normal light.

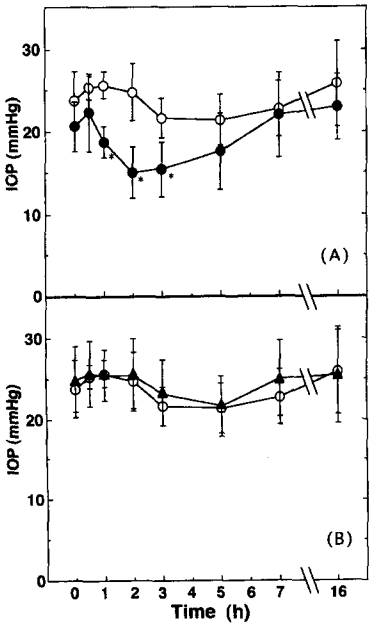

Figure 1. Anandamide (**A**: ●) and palmitylethanolamide (**B**: ▲) effects on rabbit IOP. Controls: ○. Bars: mean ± SD; n = 9. $P < 0.01$; Wilcoxon matched-pairs signed ranks test.

Results

Intraocular Pressure

Topically administered anandamide caused a statistically significant decrease in IOP at 1, 2, and 3 hours following administration (Figure 1A), compared with the IOP of eyes treated with pure mineral oil. The IOP of the contralateral eyes did not significantly decrease at any time. The maximum hypotensive effect (40%) occurred 2 hours after administration; IOP returned to baseline 7 hours after administration. Topical administration of palmitylethanolamide solution did not cause a significant decrease in IOP (Figure 1B).

Examination of IOP 2 hours following administration of varying doses (10 μg to 1 mg) of anandamide solution found a dose-dependent decrease (Figure 2). A dosage >50 μg caused a significant decrease, compared with the IOP of eyes treated with pure light mineral oil ($P = 0.05$). The hypotensive effect was maximized with 150 μg anandamide. There was no significant change in IOP of the contralateral eyes with any tested dose.

Pupil Size

There was no pupil dilation in treated eyes, compared with control eyes, during the 7 hours follow-

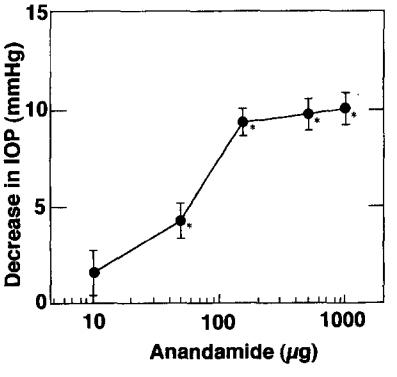

Figure 2. Anandamide dose and rabbit IOP. $P < 0.01$; 2-way ANOVA with Bonferroni's multiple comparison of group averages.

ing topical administration of anandamide solution. (Mean pupil diameter: 6.1 ± 7 mm).

Conjunctival Hyperemia and Flare

Slit-lamp examination showed that anandamide caused a noticeable conjunctival hyperemia, but no flare, 2 hours after administration of >150 μg (Figure 3a, 3b). This effect lasted for at least 3 hours. No hyperemia or flare were found after administration of palmitylethanolamide solution.

Discussion

Anandamide is a recently discovered cannabinoid receptor ligand[8] that has several cannabimimetic actions.[15] Cannabinoids have known ophthalmologic effects, including decreased IOP and conjunctival hyperemia,[16] but not pupil dilation.[17] Our study attempted to determine if anandamide also displays these effects.

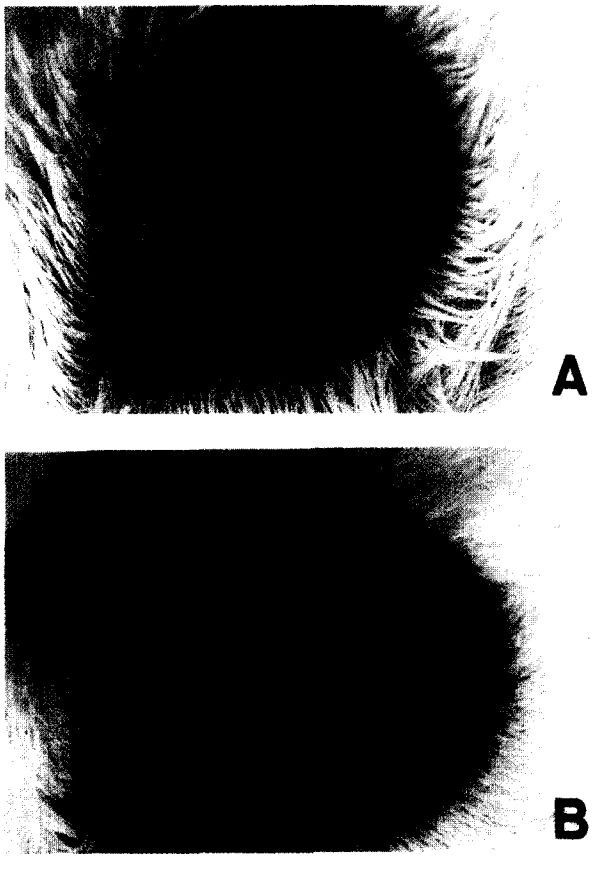

Figure 3. Conjunctival hyperemia in a rabbit eye observed 2 hours after topical administration of anandamide. (**A**) Anandamide (150 μg in 50 μL of light mineral oil) was applied to a rabbit eye. A noticeable conjunctival hyperemia was observed at 2 hours. (**B**) Fifty μL of light mineral oil was applied to the contralateral eye. No conjunctival hyperemia was observed.

Figures 1 and 2 illustrate the anandamide-induced decrease in IOP at various times and dosages. The results produced by anandamide in this study correspond to the known effects of cannabinoids. Palmitylethanolamide, with palmitate replacing the arachidonate moiety of anandamide, did not cause either hyperemia or a decrease in IOP: In spite of its structural similarity to anandamide,[10] it does not act as a cannabinoid receptor ligand. This suggests that the effect of anandamide may be mediated by a cannabinoid receptor.

Recently, cDNA for peripheral (CB2) and brain (CB1) cannabinoid receptors has been cloned.[18] CB1 is expressed in various regions of the brain, whereas CB2 is found in peripheral tissue such as the macrophages of the spleen.[18] It will be interesting to determine which subtype is expressed in the eye.

The maximum decrease in IOP produced by anandamide occurred 2 hours following administration, with a return to baseline 7 hours after administration (Figure 1). Because anandamide is hydrolyzed to arachidonic acid and ethanolamine by an amidohydrolase,[19] the IOP recovery may be the result of anandamide inactivation by this hydrolysis. We have recently identified this amidohydrolase in porcine ocular tissues.[20]

A previous report[21] noted a 25% decrease in IOP 1 hour after topical application of 50 μg Δ^9-THC in 50 μL light mineral oil. In our current study, similar topical application of 50 μg anandamide reduced the IOP 15% after 1 hour. Differences in the absorption, degradation, or affinity to the cannabinoid receptor of these two compounds may explain the difference in effect.

Just as this study was concluded, Pate and others[13,14] reported the results of various amounts of anandamide in an aqueous solution of cyclodextrin administered topically to rabbit eyes: 31.25 μg caused a decrease in IOP of 5 mm Hg but 125 μg increased the IOP. The vehicle used may have been responsible for the differences between their results and ours.

Although the mechanism by which anandamide lowered IOP is not yet clear, it has been observed that cannabinoids change IOP by altering the synthesis of an IOP modifier, prostaglandin.[22] Another report suggested that Δ^9-THC acts as a vasodilator of efferent blood vessels in the anterior uvea, leading to a decrease in IOP.[21] Cannabinoids have not been used for treatment of glaucoma, however, because they are insoluble in water and have several side-effects, including psychoactivity.[23] Our findings in this

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study may help to clarify the physiology and pathophysiology of anandamide activity in ocular tissue.

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