Effect of the Enzyme Inhibitor, Phenylmethylsulfonyl Fluoride, on the IOP Profiles of Topical Anandamides

Krista Laine,1 Kristiina Järvinen,2 David W. Pate,3 Arto Urtti,2 and Tomi Järvinen1

PURPOSE. Earlier studies have suggested that the intraocular pressure (IOP) effects of topical arachidonylethanolamide (AEA) are mediated through its fatty acid metabolite, rather than through AEA, per se. The purpose of this study was to investigate whether the topical anandamides AEA and arachidonyl propionitrileamide (APN) decrease IOP when their enzymatic degradation is prevented by phenylmethylsulfonyl fluoride (PMSF) and whether the neuronal cannabinoid (CB1) receptor mediates the IOP responses of an undegraded AEA, through the use of its specific antagonist SR141716A.

METHODS. AEA or APN were each formulated in aqueous 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) solutions and administered unilaterally to the rabbit eye (dose, 62.5 μg per rabbit). To prevent the degradation of AEA or APN, the rabbits were pretreated with a subcutaneous (SC) PMSF injection (0.22–22 mg/kg) 30 minutes before eye drop instillation. To determine whether the neuronal cannabinoid (CB1) receptor mediates the hypotensive IOP effects of undegraded AEA, the rabbits were pretreated with simultaneous SC injections of a CB1 receptor antagonist SR141716A (1.2–2.1 mg/kg) and PMSF (2.2 mg/kg) before the oculary applied AEA.

RESULTS. In the absence of PMSF, the IOP profiles of AEA and APN showed a biphasic ocular effect—that is, an initial increase in IOP followed by IOP hypotension in the treated eye. In the presence of PMSF (2.2 mg/kg for AEA and 22 mg/kg for APN), IOP profiles showed immediate IOP reduction in the treated eye. SR141716A antagonized the IOP reduction caused by the undegraded AEA.

CONCLUSIONS. These results indicate that the apparently undegraded AEA and APN decrease IOP in normotensive rabbits. AEA-induced IOP reduction in the presence of PMSF is probably mediated through a CB1 receptor. (Invest Ophthal Vis Sci. 2002;43:393–397)

It was originally observed that subjects who smoked marijuana (Cannabis sativa L.) had reduced intraocular pressure (IOP).1 This resulted in numerous studies exploring cannabinoids as possible antiglaucoma drugs.2 The endogenous cannabinoid arachidonylethanolamide (anandamide, AEA) was first isolated in porcine brain3 and was shown to bind to the neuronal CB1 receptor and subsequently to the peripheral cannabinoid receptor.4–6 AEA mimics many of the pharmacologic effects of Δ2-tetrahydrocannabinol,7 the major psychoa-
Analyzed by high-pressure liquid chromatography (HPLC). Chloride. Final drug concentration of the AEA or APN solutions was 2.5 mg/mL. Nitrogen and redissolved in aqueous 10% HP-β-CD (AEA) or 15% HP-β-CD (APN) solutions. The vehicle controls contained 10% or 20% HP-β-CD. The pH of eye drop solutions was adjusted to 7.4 with sodium hydroxide, and the solution was made isotonic to a concentration of 5 mg/mL. The solution pH was adjusted to 7.4 with aqueous sodium hydroxide, and the solution was made isotonic to a concentration of 5 mg/mL. Indomethacin, and SR141716A Solutions

Preparation and Administration of the PMSF, Indomethacin, and SR141716A Solutions

PMSF was dissolved in glycerol formal to concentrations of 3, 30, and 150 mg/mL. Depending on the test procedure, each rabbit received approximately 0.22, 2.2, and 22.0 mg/kg PMSF in a subcutaneous (SC) injection, 30 minutes before application of ocular AEA or APN, because of its low solubility in the ophthalmic vehicle.

Indomethacin was dissolved in an aqueous 20% HP-β-CD solution to a concentration of 5 mg/mL. The solution pH was adjusted to 7.4 with aqueous sodium hydroxide, and the solution was made isotonic with sodium chloride. Each rabbit in the indomethacin study received 12.5 mg (3.1–5.4 mg/kg) of indomethacin by SC injection approximately 30 minutes before the ocular AEA treatment.

An SR141716A solution (0.3 mg/mL) was prepared by dissolving the compound in a 42% HP-β-CD solution of phosphate buffer at pH 4.2. Each rabbit in the antagonism study received 4.8 mg (1.2–2.1 mg/kg) SR141716A by SC injection, 30 minutes before ocular AEA treatment. SC pretreatments with a 0.9% sodium chloride solution were used as control treatments. The single-dose levels of cannabinoid agonists and the antagonist used were selected from previous studies.24–28

IOP Measurements

To perform each test, a rabbit was placed in a plastic restraining box located in a quiet room. A single drop (25 μL) of the test solution or vehicle was instilled unilaterally into the left eye, on the upper corneoscleral limbus. The contralateral eye was left untreated. During instillation, the upper eyelid was pulled slightly away from the globe. IOP was measured using a pneumotonometer (Digilab Modular One; Bio-Rad, Cambridge, MA). Before each measurement, 1 or 2 drops of oxybuprocaine (0.06%) was applied to the cornea to eliminate discomfort. The upper and lower eyelids were then gently retracted, and the sensor was brought into contact with the center of the cornea. For each determination, at least two readings were taken from each treated (ipsilateral) and untreated (contralateral) eye, and the mean of these readings was used. IOP of the rabbits was measured at 2.1, and 0 hours before, and at 0.5, 1, 2, 3, 4, and 5 hours after, eye drop administration. IOP at the time of eye drop administration (0 hour) was used as a baseline value. All studies were set up using a masked and randomized crossover design. At least 72 hours of washout time was allowed for each rabbit between doses.

Analysis of the Data

Results are presented as a change in IOP (in millimeters of mercury) mean ± SE (n = 5–6). A one-factor analysis of variance (ANOVA) for repeated measurements was used to test the statistical significance of differences between groups. Significance in the differences of the means was tested using the Fisher’s protected least significant difference (PLSD) method at the 95% confidence level. Significance in the differences of the means between the two treatments was tested using a two-tailed paired Student’s t-test evaluated at the 95% confidence level.

RESULTS

Treated Eyes

Ocually applied AEA (62.5 μg) caused an initial increase of IOP in the treated eye followed by a statistically significant IOP reduction, when compared with a 10% HP-β-CD control solution. The maximal IOP reduction (~4.6 ± 1.4 mm Hg) occurred 3 hours after treatment (Fig. 2A). A smaller initial increase of IOP in the treated eye was also observed after ocular administration of APN (62.5 μg), a synthetic AEA analogue. A maximal decrease of IOP (~3.4 ± 1.1 mm Hg) was observed 2 hours after administration of this compound (Table 1).

To prevent AEA or APN catabolism in the eye, PMSF was administered by SC injection 30 minutes before eye drop instillation. IOP profiles of apparently undegraded AEA and APN exhibited immediate ocular hypotension after PMSF pretreatment (PMSF dose 2.2 mg/kg for AEA and 22 mg/kg for APN). The maximal IOP reduction (~3.9 ± 1.7 mm Hg) occurred 2 hours after AEA administration in the presence of PMSF (Fig. 2A). Table 1 shows that the maximal IOP reduction (~3.8 ± 1.3 mm Hg) was obtained 3 hours after ocular administration of APN with PMSF. PMSF pretreatment at a dose of 0.22 mg/kg for AEA and 2.2 mg/kg for APN did not eliminate the initial in-
TABLE 1. IOP Changes in the Treated Eyes of Rabbits after Administration of Test Compounds

<table>
<thead>
<tr>
<th>Ocular Treatment</th>
<th>Subcutaneous Pretreatment</th>
<th>Time after Eye Drop Application (h)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
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<tr>
<td>APN</td>
<td>0.9% NaCl</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>APN</td>
<td>PMSF 2.2 mg/kg</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>APN</td>
<td>PMSF 22.0 mg/kg</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>20% HP-β-CD</td>
<td>0.9% NaCl</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>AEA</td>
<td>0.9% NaCl</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>AEA</td>
<td>PMSF 0.22 mg/kg</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>AEA</td>
<td>PMSF 2.2 mg/kg</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>10% HP-β-CD</td>
<td>0.9% NaCl</td>
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<tr>
<td>10% HP-β-CD</td>
<td>PMSF 0.22 mg/kg</td>
<td>0.0 ± 0.0</td>
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<td>10% HP-β-CD</td>
<td>PMSF 2.2 mg/kg</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>10% HP-β-CD</td>
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<tr>
<td>AEA</td>
<td>PMSF 2.2 mg/kg</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>10% HP-β-CD</td>
<td>PMSF 2.2 mg/kg + SR141716A</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Data are mean mm Hg ± SE (n = 5–6). Dose was 62.5 μg/25 μL.

*Statistically significant compared with vehicle treatment (10% HP-β-CD or 20% HP-β-CD with 0.9% NaCl; P < 0.05; ANOVA, Fisher’s PLSD).

FIGURE 2. IOP changes (mean ± SE, n = 5) in treated (A) and untreated (B) eyes of normotensive rabbits after unilateral ocular administration of vehicle (25 μL). AEA (62.5 μg) after pretreatment with NaCl (Δ) or PMSF 2.2 mg/kg (○). Significantly different from vehicle-treated group (P < 0.05, ANOVA, Fisher’s PLSD).

Unilateral ocular administration of AEA (Fig. 2B) or APN (data not shown), with or without PMSF pretreatment, did not significantly affect IOP in untreated eyes, compared with the vehicle treatment. However, a statistically significant increase of IOP was observed in contralateral eyes when SC SR141716A and PMSF (2.2 mg/kg) were simultaneously administered with either the ophthalmic vehicle or AEA (Fig. 3B).

### Discussion

The endogenous cannabinoid AEA is hydrolyzed rapidly to arachidonic acid and ethanolamine by FAAH enzyme activity. The formed arachidonic acid may serve as a precursor for COX-catalyzed biosynthesis of prostanoids (Fig. 1). It is suggested that the prostaglandin synthesis may play a role for mediating the IOP effects of AEA in the absence of an FAAH inhibitor (Table 1, Fig. 2A), because the IOP responses of AEA and arachidonic acid (i.e., initial increase and subsequent ocular hypotension) can be eliminated by the COX inhibitor indomethacin.26

In this study, prevention of arachidonic acid formation from AEA and APN and subsequent synthesis of prostanoid derivatives was attempted through the use of the FAAH inhibitor PMSF. This was intended to evaluate whether degraded AEA and its synthetic analogue APN decreases IOP in normotensive rabbits. In the presence of PMSF, the IOP profiles of AEA and APN exhibited only a hypotensive phase, suggesting that neither AEA nor APN was degraded. Indeed, prostaglandin syn-

Unilateral ocular administration of AEA (62.5 μg) and SC PMSF (22 mg/kg). The indomethacin pretreatment did not inhibit the IOP reduction effects caused by AEA in the presence of PMSF (Table 2).

The CB1 receptor antagonist, SR141716A, was used to determine whether IOP reduction effects caused by the degraded AEA were mediated through a CB1 receptor. In the presence of simultaneous SR141716A and PMSF (2.2 mg/kg) SC pretreatments, the AEA decrease of IOP was eliminated (Fig. 3A). SC pretreatment by SR141716A (4.8 mg) and PMSF (2.2 mg/kg) and administration of the ophthalmic control vehicle (10% HP-β-CD) induced a statistically significant IOP increase in the treated eye compared with the effects of SC NaCl before ophthalmic vehicle administration (10% HP-β-CD; Table 1).
thesis through the COX pathway seems not to be involved in the IOP effects of test compounds in the presence of PMSF, because the COX inhibitor (indomethacin) pretreatment had no influence on the IOP reduction effects of AEA in the presence of PMSF (Table 2). These results suggest that the ocular hypotension seen after ocular administration of AEA or APN, in the presence of the FAAH inhibitor PMSF, is due to unmetabolized AEA or APN. However, it should be noted that PMSF is also a potent inhibitor of cholinesterases and other serine proteases and thus is nonselective for FAAH.

A specific CB1 cannabinoid receptor antagonist, SR141716A, was used to examine whether the IOP reduction induced by AEA in the presence of PMSF is mediated through CB1 receptors. In earlier studies, SR141716A has failed to antagonize the ocular effects of AEA. In this study, in the presence of the FAAH inhibitor, PMSF, hypotensive IOP effects of AEA were inhibited by the CB1 receptor antagonist, and an IOP increase was observed in treated and untreated eyes. Similar bilateral hypertension was observed when the CB1 receptor antagonist and PMSF were administered in conjunction with the ocular vehicle. This observation is consistent with earlier studies and supports the hypothesis that the CB1 receptor may be involved with the physiological control of IOP. SR141716A may increase IOP by acting as an antagonist for the endogenous cannabinoid receptor agonist, blocking its tonic regulatory effects on IOP. SR141716A has also been reported to act as an inverse agonist under certain test conditions.

In conclusion, this study reports for the first time that ocularly administered AEA, an endogenous ligand of the CB1 receptor, and its synthetic analogue APN reduced IOP without the initial IOP hypertensive phase if their enzymatic degradation was prevented by the enzyme inhibitor PMSF. The IOP reduction induced by AEA in the presence of PMSF was mediated through a CB1 receptor, suggesting that the endocannabinoid system may have a physiological role in the regulation of ocular tension.

Acknowledgments

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References


