N-arachidonylethanolamide-Induced Increase in Aqueous Humor Outflow Facility

Ya Fatou Njie,[1] *Zhuanhong Qiao,*[1] *Zhen Xiao,*[1,2] *Wei Wang,*[2] *and Zhao-Hui Song*[1]

Purpose. To study the effects of *N*-arachidonylethanolamide (anandamide [AEA]) on aqueous humor outflow and to investigate the existence and activity of fatty acid amide hydrolase (FAAH), an AEA metabolic enzyme in trabecular meshwork (TM) tissue.

Methods. The effects of AEA on aqueous humor outflow were measured using a porcine anterior segment–perfused organ culture model. Western blot analysis was used to study the expression of FAAH, and a thin-layer chromatography–based approach was used to measure the enzymatic activity of FAAH in TM tissue.

Results. Administration of AEA caused a transient enhancement of aqueous humor outflow facility. In the presence of 100 nM URB597, an FAAH inhibitor, the effect of 10 nM AEA on outflow facility was prolonged by at least 4 hours. The AEA-induced enhancement of outflow facility was blocked by SR141716A, a CB1 antagonist, and was partially blocked by SR144528, a CB2 antagonist. In Western blot studies, positive signals were detected on TM tissues with an anti-FAAH antibody. In the enzyme activity studies, the enzymatic activity of AEA hydrolysis was detected in TM tissues, and this activity was reduced with the addition of 100 nM URB597.

Conclusions. Results from this study demonstrate that the administration of AEA increases aqueous humor outflow facility and that this effect of AEA involves CB1 and CB2 cannabinoid receptors. In addition, this study reveals the existence and the activity of FAAH, an AEA-metabolizing enzyme, in the TM tissues. (*Invest Ophthalmol Vis Sci.* 2008;49:4528–4534) DOI: 10.1167/iovs.07-1537

The major sites of actions for cannabinoid ligands are CB1 and CB2 cannabinoid receptors.[1,2] The CB1 receptor is located in the central nervous system and in peripheral tissues, whereas the CB2 receptor is distributed primarily in the periphery.[1–4]

N-arachidonylethanolamide (anandamide [AEA]) was first isolated from porcine brain[5] and was shown to bind to the CB1 and CB2 receptors, with a higher affinity for the former.[6,7] This endogenous ligand has been reported to mimic many of the pharmacologic effects of Δ^9-tetrahydrocannabinol, the major psychoactive constituent of marijuana.[8,9]

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Since the original paper by Hepler and Frank[10] in the early 1970s reported that subjects who smoked marijuana had reduced intraocular pressure, numerous studies exploring cannabinoids as possible antiglaucoma agents have been conducted.[11–13] The discovery of CB1 and CB2 cannabinoid receptors in the eye supports a possible role of cannabinoid receptors in controlling IOP.[14–17] Previously, several studies have shown that cannabinoid receptors are involved in the IOP-lowering effects of cannabinoid agonists.[18–20]

Topical administration of AEA to rabbit eyes has been shown to lower IOP in a dose-dependent manner.[21,22] However, the mechanism by which AEA produces its IOP-lowering effects is not yet known. IOP is maintained by a dynamic balance between the secretion of aqueous humor by the ciliary body and the outflow of aqueous humor by the conventional (trabecular meshwork [TM]) and uveoscleral route. The TM is the major site for aqueous humor outflow and thus is important for the regulation of IOP. In our recent studies, we demonstrated that the administration of CB1-selective or CB2-selective cannabinoid agonists enhances aqueous humor outflow through the conventional (TM) pathway and that this aqueous humor outflow-enhancing effect involves the CB1 and CB2 cannabinoid receptors.[16,17] Interestingly, in a previous study, endogenous AEA has been detected in the TM tissues of human eyes.[14] Based on the results of these previous studies and the fact that AEA is a nonselective cannabinoid agonist with affinity for CB1 and CB2 cannabinoid receptors, we hypothesized in this study that by acting on cannabinoid receptors, AEA may enhance aqueous humor outflow in the anterior segments.

AEA has a short duration of action in vivo.[23–27] The cellular actions of AEA is terminated by the enzyme fatty acid amide hydrolase (FAAH),[23–27] which hydrolyzes it to arachidonic acid and ethanolamine (Fig. 1). The presence of FAAH has been reported in a variety of ocular tissues.[28–30] However, thus far the existence and activity of FAAH have not yet been examined in the TM tissues. Thus a second hypothesis of this study was that active FAAH protein may be present in the TM tissues.

First, we first investigated whether AEA has a direct effect in modulating aqueous humor outflow facility in the porcine anterior segments and whether this effect of AEA is mediated by cannabinoid receptors. Second, we examined the presence and activity of the AEA metabolic enzyme FAAH in the TM tissues and its involvement in regulating AEA-induced enhancement of aqueous humor outflow facility. Third, we investigated the effects of URB597, a specific and potent inhibitor of FAAH,[31,32] on aqueous humor outflow.

Materials and Methods

Materials

AEA was purchased from Tocris Cookson, Inc. (Ellisville, MO). [^{3}H]AEA was purchased from American Radiolabeled Chemicals (St. Louis, MO). The anti-FAAH antibody and URB597 were purchased from Cayman Chemical (Ann Arbor, MI). Cannabinoid receptor antagonists SR141716A and SR144528 were obtained from the National Institute of Drug Abuse (Rockville, MD). Precoated silica gel 60 glass plates for thin-layer chromatography (20 × 20 cm, 0.25-mm thickness) were purchased from Fisher Scientific (Pittsburgh, PA).

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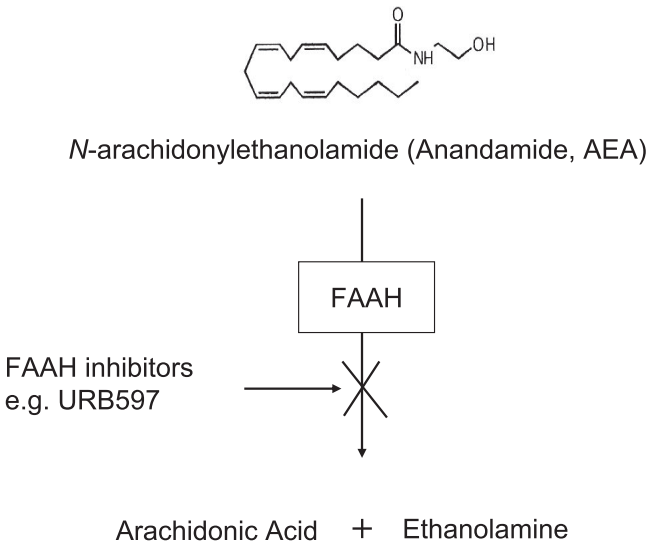

FIGURE 1. Chemical structure and hydrolysis pathway of *N*-arachidonylethanolamide (anandamide [AEA]).

Porcine Anterior Segment Perfusion Model

A previously published protocol[33] was followed for the anterior segment–perfused organ culture model. Fresh porcine eyes were obtained from a local slaughterhouse within 30 minutes of decapitation. Porcine anterior segment explants, constituting the intact cornea, the undisturbed TM, and a 2- to 5-mm rim of sclera with the ciliary body and iris gently removed, were mounted in a standard perfusion culture apparatus and were perfused with Dulbecco modified Eagle medium (DMEM) for 1 day while outflow stabilized, with a constant perfusion head of 10 cm (approximately 7.35 mm Hg). Only those explants that stabilized between 1.5 and 8 μL/min at 7.35 mm Hg were used. Cultures were maintained at 37°C with 5% CO_2 and 95% air. It had been shown previously that in this model, outflow is through the TM, and flow rates are physiological.[33] At the end of the perfusion study, the anterior segments were perfusion fixed at 7.35 mm Hg constant pressure with 4% paraformaldehyde for 1 hour. Anterior segments were then removed from the perfusion chamber, and 2- to 3-mm–wide wedges from each quadrant containing outflow tissues were cut and immersed in 10% formalin for 1 hour and then in 70% alcohol overnight. Subsequently, tissues were embedded in paraffin and stained with hematoxylin and eosin (HE). The viability of outflow pathway tissues was evaluated by light microscopy. A representative photograph of ocular anterior segments with normal TM morphology has been published by us previously.[16] Perfusion studies were regarded as invalid, and data were discarded if more than one quadrant per eye had unacceptable morphologic findings, such as excessive TM cell loss and denudation of trabecular beams. Approximately 20% of eyes were rejected based on the morphologic and perfusion rate criteria described. After establishing a baseline outflow, AEA was introduced by exchanging the perfusion chambers with drug-containing medium. Anterior segments were then perfused continuously with drug-containing medium for 5 hours, and the outflow facility was monitored. Vehicle control was run in parallel. For the antagonist and enzyme inhibitor studies (Fig. 2), the antagonist or enzyme inhibitor (sometimes both) was applied to the perfusion medium 30 minutes before treatment with AEA and was present throughout the treatment. For the high-pressure studies, the anterior segments were perfused using a constant perfusion head of 20 cm (approximately 15 mm Hg), and the enzyme inhibitor URB597 was administered after stabilization and monitored for 5 hours. Vehicle was also run in parallel.

Outflow facility was calculated as the ratio of the rate of flow of perfusate (μL/min) to the steady state perfusion pressure (mm Hg). Drug effects were evaluated in each eye as the percentage change of outflow facility in drug-treated eyes over predrug baseline outflow facility. Approximately 10 unmatched eyes were used for each group of treatment. Data were presented as mean ± SE and were plotted as change in outflow facility versus time (in minutes) using Prism software (Graph Pad, San Diego, CA).

Western Blot Analysis

TM tissues were isolated from fresh porcine eyes by blunt dissection and were sonicated in lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin). Samples were centrifuged at 20,000*g* for 10 minutes, the supernatant obtained was incubated with 2× Laemmli sample buffer at room temperature for 20 minutes, and proteins were resolved on a 10% SDS-polyacrylamide gel using a minigel electrophoresis system (Invitrogen, Carlsbad, CA). Protein bands were transferred onto a nitrocellulose membrane for immunoblotting. Nitrocellulose membranes were blocked with 5% nonfat dried milk in TBS-T (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, and 0.3% Tween 20) buffer for 1 hour and then incubated overnight at 4°C with the anti-FAAH polyclonal antibody (Cayman Chemical). Subsequently, the membranes were washed twice for 10 minutes each time with TBS-T buffer and incubated with anti-rabbit horseradish peroxidase secondary antibody for 1 hour at room temperature. The membranes were then washed three times with TBS-T buffer for 10 minutes each time, and the antibody-recognized protein bands were visualized by an enhanced chemiluminescence detection kit (ECL; Amersham Biosciences, Piscataway, NJ).

Fatty Acid Amide Hydrolase Assay

Isolated TM tissue from porcine eyes was sonicated in ice-cold 50 mM Tris-HCl (pH 9) with a converter (Ultrasonic Converter; Fisher Scientific) and centrifuged at 300*g* for 5 minutes to remove debris. Protein concentration was then determined by the method of Bradford (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. The assay for the FAAH activity was carried out using a previously established method with minor modifications.[28] The TM homogenate was incubated with 5 μM [^{3}H] AEA at 37°C for 30 minutes in 50 mM Tris-HCl (pH 9). Reaction was terminated by the addition of 400 μL of a mixture of ethylether/methanol/1 M citric acid (30:4:1 vol/vol) and 20 μL of 1*N*

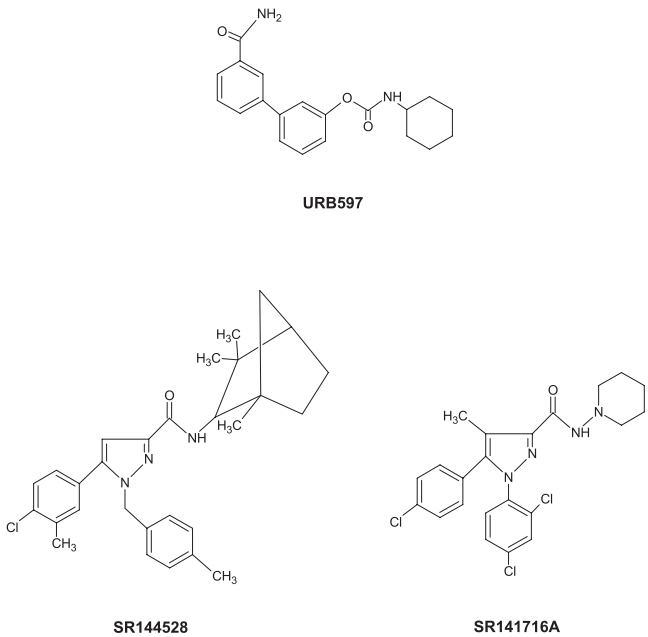

FIGURE 2. Chemical structures of the FAAH inhibitor URB597, CB1 antagonist SR141716A, and CB2 antagonist SR144528.

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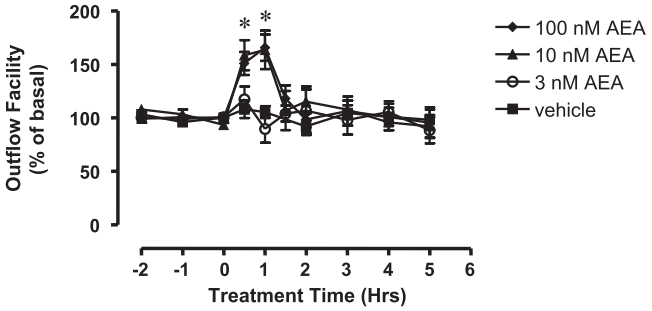

FIGURE 3. Effects of AEA on aqueous humor outflow facility. Two different concentrations of AEA were used. Results are expressed as mean ± SE; $n = 10$. *Significant differences between 10 nM AEA, 100 nM AEA, and vehicle ($P < 0.05$, ANOVA with Neuman-Keuls posttest).

HCl. Phases were then separated by centrifuging for 1 minute at 800*g,* and the ethereal extract was spotted on a silica gel 60-glass plate and subjected to thin-layer chromatography using a solvent system of chloroform/methanol/ammonium hydroxide (80:20:2, vol/vol) for 45 minutes at room temperature. Lipids were visualized by exposure to iodine vapors, spots corresponding to AEA and its metabolic product arachidonic acid were scraped off the plate, and the radioactivity was quantified by liquid scintillation counting. Assays were performed in triplicate and were repeated three times. For the enzyme assay with inhibitor, the homogenate was preincubated with the FAAH enzyme inhibitor URB597 for 30 minutes at 4°C before the addition of AEA, and the enzyme activities were assayed as described.

Data Analyses

For anterior segment perfusion studies, results are presented as a change in outflow facility (percentage of basal) mean ± SE. One-way ANOVA or unpaired two-tailed Student's *t*-tests were used to compare the data points of the treatment groups. The level of significance was chosen as $P < 0.05$. Data points for FAAH assays were expressed as mean ± SE, plotted using Graph Pad Prism, and analyzed with unpaired two-tailed Student's *t*-tests. The level of significance was chosen as $P < 0.05$.

RESULTS

Effects of AEA on Aqueous Humor Outflow Facility

Aqueous humor outflow facility studies were performed using the porcine anterior segment perfused organ culture model. Basal aqueous humor outflow rates were 2.05 ± 0.18 μL/min and 3.61 ± 0.28 μL/min, at the perfusion heads of 10 cm and 20 cm, respectively. This corresponded to outflow facilities of 0.28 ± 0.02 μL/min/mm Hg and 0.24 ± 0.02 μL/min/mm Hg, at 7.35 mm Hg and 15 mm Hg, respectively. AEA has been shown in previous studies to produce pharmacologic effects at low to high nanomolar concentrations.[6–9] As shown in Figure 3, the application of 3 nM AEA, an endogenous cannabinoid agonist, had no effect on outflow facility. However, the application of 10 and 100 nM AEA caused a transient enhancement of aqueous humor outflow at 0.5 to 1 hour after treatment when compared with vehicle. AEA at 100 nM had an outflow-enhancing effect no greater than that at 10 nM (Fig. 3).

AEA is hydrolyzed rapidly to arachidonic acid and ethanolamine by FAAH (Fig. 1).[23–27] Previous studies on IOP have shown that inhibition of this enzyme prolongs the IOP-lowering effects of AEA.[18] URB597 (Fig. 2), a selective and potent inhibitor FAAH activity, was tested in this study to determine whether it had an effect on AEA-induced enhancement of outflow facility. A URB597 concentration of 100 nM was chosen based on the known pharmacologic effects of this inhibitor.[31,32] Perfused ocular anterior segments were pretreated with 100 nM URB597 for 30 minutes before the administration of 10 nM AEA and were continuously perfused with AEA for 5 hours. In the presence of URB597, AEA induced-increase in outflow facility was prolonged; this effect lasted for at least 5 hours (Fig. 4). Compared with vehicle, 100 nM URB597 alone had no significant effect on outflow facility (see Fig. 9A).

Effects of CB1 and CB2 Antagonists on AEA-Induced Enhancement of Outflow Facility

SR141716A, an antagonist selective for CB1 receptor (Fig. 2),[34] was used to determine whether the increase in outflow facility caused by AEA was mediated through the CB1 cannabinoid receptor. In the presence of 1 μM SR141716A, the outflow-enhancing effects of 10 nM AEA plus 100 nM URB597 was completely blocked (Fig. 5A). SR141716A alone had no effect on outflow facility (Fig. 5B). SR144528, an antagonist selective for CB2 receptor antagonist (Fig. 2),[35] was used to determine whether the AEA-induced increase in outflow facility involves the CB2 cannabinoid receptor. As shown in Figure 6A, in the presence of 1 μM SR144528, the outflow-enhancing effects of 10 nM AEA plus 100 nM URB597 was partially antagonized. Figure 6B shows that SR144528 alone had no significant effect on outflow facility compared with vehicle treatment.

Identification of Fatty Acid Amide Hydrolase Proteins in Trabecular Meshwork Tissues

Western blot analysis was performed to determine whether FAAH, the metabolizing enzyme for AEA, is expressed in TM tissues. As shown in Figure 7, a protein band with a molecular mass of approximately 65 kDa was detected in the TM tissue homogenate with the specific anti-FAAH antibody but not with the same antibody preabsorbed with peptide antigen.

Metabolism of AEA by Fatty Acid Amide Hydrolase in Trabecular Meshwork Tissues

A homogenate of TM tissues was incubated with [^{3}H]AEA, and the product of enzymatic hydrolysis of [^{3}H]AEA was separated by thin-layer chromatography. This was done to test whether the enzyme FAAH is involved in the metabolism of AEA in these tissues. With the use of thin-layer chromatography, it was observed that [^{3}H] AEA was rapidly hydrolyzed and the main metabolic product was a compound comigrating with the free fatty acid, arachidonic acid. As shown in Figure 8, the rate of

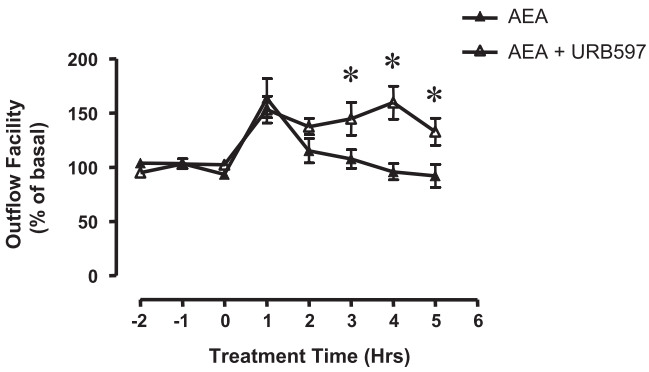

FIGURE 4. Effects of URB597 on AEA-induced enhancement of aqueous humor outflow facility. Anterior segments were treated with 100 nM URB597 for 30 minutes before treatment with 10 nM AEA + 100 nM URB597 for 5 hours. Results are expressed as mean ± SE; $n = 10$. *Significant differences between AEA alone and AEA + URB597 groups ($P < 0.05$, *t*-test).

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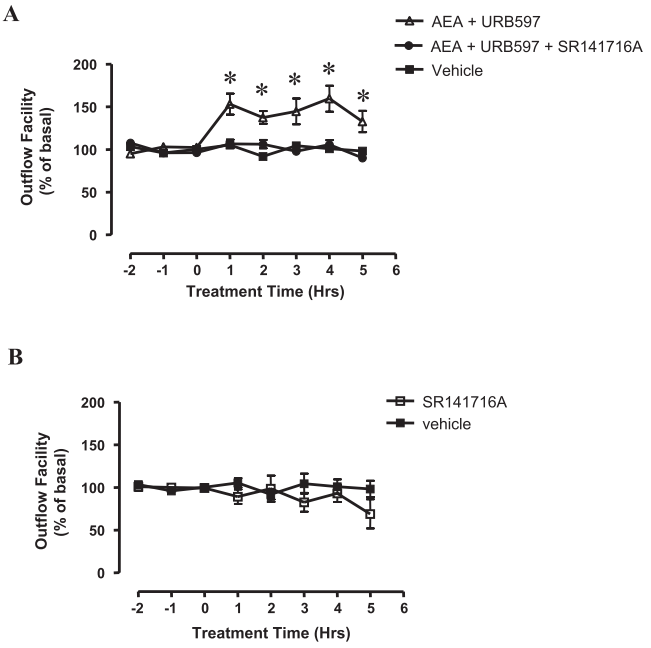

FIGURE 5. Antagonism of AEA-induced increase of aqueous humor outflow facility by CB1 antagonist SR141716A. Anterior segments were treated with 1 μM SR141716A + 100 nM URB597 for 30 minutes before treatment with 10 nM AEA + 100 nM URB597 + 1 μM SR141716A for 5 hours (**A**). Results are expressed as mean ± SE; n = 10. *Significant differences between AEA + URB597 and AEA + URB597 + SR141716A groups ($P < 0.05$, ANOVA with Neuman-Keuls posttest). SR141716A alone did not have any significant effect on outflow (**B**).

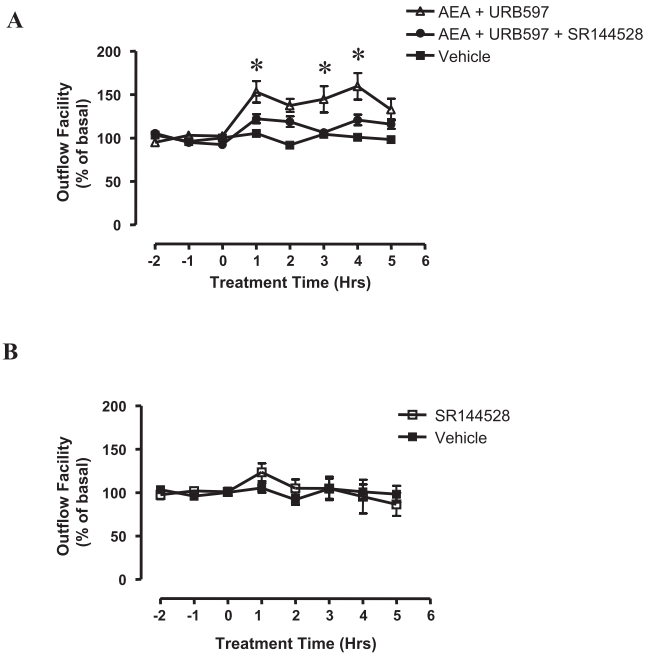

FIGURE 6. Antagonism of AEA-induced increase of aqueous humor outflow facility by CB2 antagonist SR144528. Anterior segments were treated with 1 μM SR144528 + 100 nM URB597 for 30 minutes before treatment with 10 nM AEA + 100 nM URB597 + 1 μM SR144528 for 5 hours (**A**). Results are expressed as mean ± SE; n = 10. *Significant differences between AEA + URB597 and AEA + URB597 + SR144528 groups ($P < 0.05$, ANOVA with Neuman-Keuls posttest). SR144528A alone did not have any significant effect on outflow (**B**).

hydrolysis of [^{3}H]AEA was protein concentration-dependent. The enzyme activity of AEA hydrolysis was 0.15 ± 0.04 nmol min^{-1} mg^{-1} protein (mean ± SE). URB597, a selective and potent inhibitor of FAAH, significantly inhibited the hydrolysis of AEA (Fig. 8). In the presence of URB597, the hydrolysis activity of FAAH was reduced by 86.6% ± 5.3%.

Effects of URB597 on Aqueous Humor Outflow Facility under Elevated Pressure

Studies have reported that endocannabinoids are selectively and transiently elevated during certain pathologic conditions[36–39] and that inhibitors of endocannabinoid inactivation may potentiate their actions.[18,31,32] Therefore, we investigated the effects of several concentrations of URB597 at two different perfusion pressures. As mentioned earlier, it has been reported that in the anterior segment perfusion model, flow rates are physiological at a pressure of 7.35 mm Hg.[33] Assuming an episcleral venous pressure of 8 to 10 mm Hg, this perfusion pressure would simulate an in vivo IOP of 15.35 to 17.35 mm Hg. Consequently, increasing the perfusion pressure to 15 mm Hg would mimic an in vivo IOP of 23 to 25 mm Hg. As shown in Figure 9A, at a perfusion pressure of 7.35 mm Hg, none of the concentrations of URB597 had any significant effects on aqueous humor outflow facility. In contrast, URB597 produced a concentration-dependent enhancement of aqueous humor outflow facility when the perfusion pressure for the porcine anterior segment was raised to 15 mm Hg (Fig. 9B).

DISCUSSION

The presence of an endogenous cannabinoid system—endocannabinoid ligands,[14,40,41] cannabinoid receptors,[14–17,42] and endocannabinoid-metabolizing enzymes,[28–30] in ocular tissues—suggests a possible involvement of this system in many functions of the eye, including the regulation and maintenance of IOP. Previous studies have reported that cannabinoids are effective in reducing IOP and that their IOP-lowering effects are mediated at least in part by ocular cannabinoid receptors.[10–13,18–22] In a recent study, we reported that the administration of noladin, an endogenous cannabinoid agonist selec-

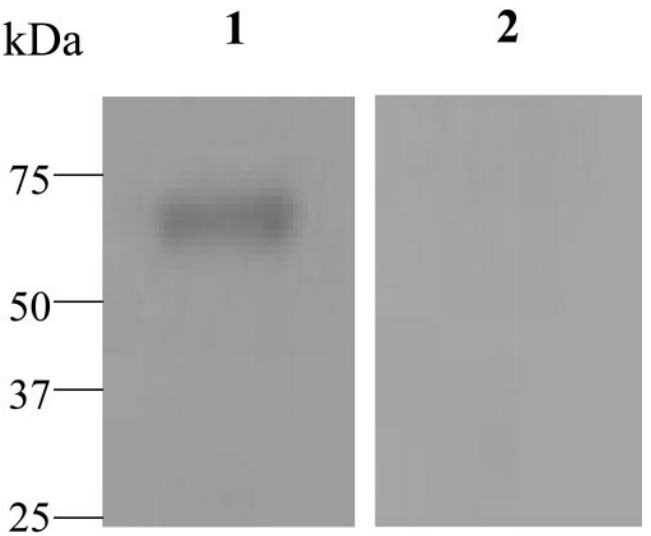

FIGURE 7. Expression of FAAH protein on trabecular meshwork tissues. Western blot analysis. *Lane 1*: with primary anti-FAAH antibody; *lane 2*: with primary anti-FAAH antibody preabsorbed with the peptide antigen. Western blot is a representative of three independent experiments.

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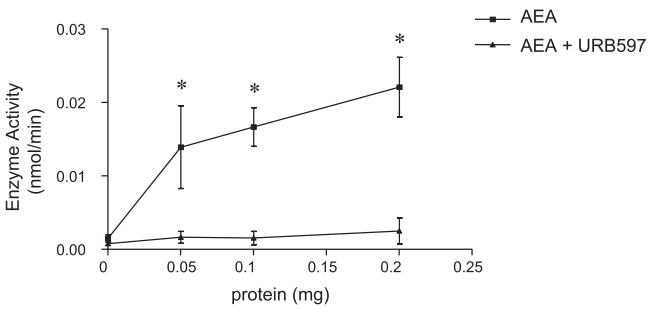

FIGURE 8. Identification of FAAH activity of trabecular meshwork tissues. Trabecular meshwork homogenates were assayed for enzymatic hydrolysis of AEA in the absence (■) or the presence (▲) of 100 nM URB597. Different amounts of the homogenates were allowed to react with 5 μM [^{3}H]AEA. The assay was repeated three times (each time in triplicate), and the mean ± SE is shown. *Significant differences in enzymatic hydrolysis of AEA between the two groups (with or without 100 nM URB597; $P < 0.05$, *t*-test).

tive for CB1, increases aqueous humor outflow facility by acting through CB1 cannabinoid receptors.[17] In another study, we showed that application of JWH015, a cannabinoid agonist selective for CB2, also increases aqueous humor outflow facility and that this outflow-enhancing effect involves CB2 receptors.[16] Previously, AEA has been demonstrated to lower IOP.[18,21,22] However, until the present study, the effect of AEA on aqueous humor outflow had not yet been reported.

In this study, we used porcine perfused anterior segments to examine the effect of AEA on aqueous humor outflow facility. Porcine eyes have been used widely as experimental models for human eyes because they have considerable anatomic and physiological similarities.[43–46] Furthermore, their availability and comparable size to the human eye have made it an established model for studying various aspects of the aqueous humor outflow system.[43–46] Application of AEA alone had a transient effect on outflow (Fig. 3). It has been reported that AEA is synthesized and released "on demand" in certain physiological and pathologic conditions.[36–39] After its biosynthesis and release, AEA is apparently transported to cells by a carrier-mediated uptake mechanism. Once in the cells, its actions are terminated by FAAH[23–27] (Fig. 1). Based on this concept, we hypothesized that the transient effect of AEA on aqueous humor outflow facility might be attributed to its metabolism by FAAH in the TM tissues.

This hypothesis is supported by our data using URB597, a potent inhibitor for FAAH. URB597 is an *O*-arylcarbamate that has been reported to be an irreversible and highly selective inhibitor for the FAAH enzyme. In other words, it can enhance endogenous AEA signaling without directly interacting with cannabinoid receptors or the transporter proteins.[31,32] In the presence of URB597, the aqueous humor outflow-enhancing effects of AEA were prolonged by at least 4 hours (Fig. 4). These data are consistent with the report of Laine et al.,[18] which demonstrated that in the presence of phenylmethylsulfonyl fluoride (PMSF), a nonselective serine protease inhibitor that inhibits the hydrolysis of AEA, the IOP-lowering effects of AEA were potentiated.

AEA is known to exhibit different binding properties and intrinsic activities at CB1 and CB2 receptors.[6,7] It is a partial agonist at the CB1 and CB2 receptors but has a slightly higher affinity and much greater efficacy for the CB1 receptor.[6,7] To determine whether the AEA-induced enhancement of aqueous humor outflow facility in the presence of URB597 is mediated through CB1 receptors, we used SR141716A, a selective antagonist for CB1. The enhanced outflow facility induced by AEA plus URB597 was completely antagonized by SR141716A (Fig. 5). Similarly, we used a CB2-selective antagonist SR144528 to examine whether the CB2 receptor is involved in AEA-induced increases in aqueous humor outflow. SR144528 partially blocked the AEA-induced increase in outflow (Fig. 6). These observations indicate that AEA-induced increases in outflow facility are mediated primarily by the CB1 cannabinoid receptor and partially by the CB2 receptor. Our results using the CB1 antagonist are consistent with a previous study showing that IOP reduction induced by AEA in the presence of PMSF was mediated through CB1 receptors.[18] It is not clear why the outflow-enhancing effects of AEA can be blocked completely by the CB1 antagonist and partially by the CB2 antagonist. One explanation could be that AEA has higher affinity and efficacy for CB1; thus, its effects may be mediated primarily by CB1 receptors. Because of the limited sensitivity of our perfusion studies, in the presence of the CB1 antagonist, the residue effects mediated by the CB2 may be not detectable. On the contrary, in the presence of the CB2 antagonist, the outflow-enhancing effects of CB1 are strong enough to be detected by our perfusion studies.

Many studies have reported the expression of mRNA and proteins of FAAH and its enzymatic activities in various tissues.[23–27,29,47,48] In addition, it has been demonstrated that the anatomic distribution of FAAH mRNA, protein, and activity correlates with the distribution of cannabinoid receptors.[47,48] Although FAAH activity has been reported in various ocular tissues of different species, including porcine retina, iris, choroids, optic nerve, rat retina and bovine retina,[28–30] FAAH presence and activity have not yet been studied in TM tissues until the present study. To test our hypotheses that the AEA metabolic enzyme FAAH is present in the TM and that it is involved in the degradation of AEA in this tissue, we performed Western blot analysis and measured FAAH activity using a TLC-based method. We demonstrated for the first time, in the present study, the presence of FAAH in the TM tissues (Fig. 7).

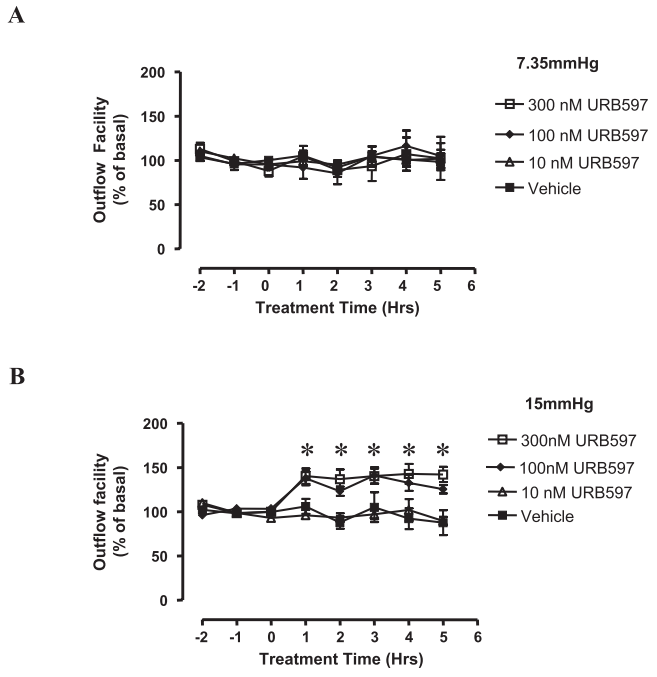

FIGURE 9. Effects of URB597 on aqueous humor outflow facility. URB597-treated anterior segments were perfused at 7.35 mm Hg (**A**) and 15 mm Hg (**B**). Results are expressed as mean ± SE; $n = 10$. *Significant differences in aqueous humor outflow facility between 100 nM and 300 nM URB597-treated and vehicle-treated anterior segments ($P < 0.05$, ANOVA with Neuman-Keuls posttest).

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Furthermore, we showed that this tissue has potent AEA hydrolyzing activity that was inhibited by treatment with URB597 (Fig. 8). These findings further support the notion that the endocannabinoid ligand AEA is metabolized in the TM tissue by the FAAH enzyme.

In view of our data on the prolongation effect of URB597 on AEA-induced enhancement of aqueous humor outflow, we reasoned that under elevated IOP, endogenous cannabinoids might be activated on demand and that URB597 may affect aqueous outflow by inhibiting the degradation of endogenous cannabinoids such as AEA. In line with this hypothesis, we observed that application of URB597 to the perfused anterior segment organ culture under higher pressure (15 mm Hg) induced a concentration-dependent increase in outflow facility (Fig. 9B). In contrast, under normal perfusion pressure (7.35 mm Hg), URB597 was not effective on aqueous humor outflow (Fig. 9A). These results indicate that under elevated IOP, endocannabinoids such as AEA might be produced by the anterior segment tissues to compensate for the increased pressure.

In conclusion, this study reports for the first time that administration of the endocannabinoid ligand AEA increases aqueous humor outflow facility; this effect of AEA involves both the CB1 and the CB2 cannabinoid receptors. In addition, this study demonstrates the existence and the activity of FAAH, an important endocannabinoid-metabolizing enzyme, in the TM tissues. Furthermore, this study indicates that by inhibiting the degradation of AEA and other endocannabinoids, specific FAAH inhibitors such as URB597 may provide us with new hope for effectively lowering IOP by enhancing aqueous humor outflow.

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