Noladin Ether Acts on Trabecular Meshwork Cannabinoid (CB1) Receptors to Enhance Aqueous Humor Outflow Facility

Ya Fatou Njie, Akhilesh Kumar, Zhuanhong Qiao, Lichun Zhong, and Zhao-Hui Song

Purpose. To study the effects of 2-arachidonyl glyceryl ether (noladin ether), an endocannabinoid ligand selective for cannabinoid (CB)1 receptor, on aqueous humor outflow facility, to investigate the involvement of trabecular meshwork CB1 receptors and the p42/44 MAP kinase signaling pathway and to explore the cellular mechanisms of noladin ether–induced changes of outflow facility.

Methods. The effects of noladin ether on aqueous humor outflow facility were measured in a porcine anterior-segment–perfused organ culture model. The expression of CB1 receptors on cultured porcine trabecular meshwork cells and the coupling of these receptors to p42/44 MAP kinase was determined by immunofluorescence microscopy and Western blot analysis. Both Western blot and zymography were used to monitor the effects of noladin ether on matrix metalloproteinase (MMP)-2. In morphologic studies, AlexaFluor 488-labeled phalloidin staining was used to examine actin filament, and immunohistochemistry with anti-paxillin antibodies was used to detect focal adhesions.

Results. Within 1 hour after adding 3, 30, or 300 nM of noladin ether, the aqueous humor outflow facility increased concentration dependently. The effect of 30 nM of noladin ether was completely blocked by SR141716A, a selective CB1 antagonist. Positive signals were detected on cultured porcine trabecular meshwork cells with an anti-CB1 antibody in immunofluorescence microscopy and Western blot studies. Treatment of trabecular meshwork cells with 30 nM of noladin ether activated p42/44 MAP kinase, whereas pretreatment with SR141716A blocked the p42/44 MAP kinase-activating effects of noladin ether. In addition, the enhancement of outflow facility induced by noladin ether was blocked by pretreatment of porcine anterior segments with PD98059, an inhibitor of p42/44 MAP kinase pathway. Furthermore, noladin ether treatment caused rounding of trabecular meshwork cells, and there was a decrease of actin stress fibers, as well as a decrease in focal adhesions. These noladin ether-induced morphologic changes were also blocked by SR141716A and PD98059.

Conclusions. The results demonstrate for the first time that administration of noladin ether, an endocannabinoid agonist selective for the CB1 receptor, increases aqueous humor outflow facility. The data also show that noladin ether–induced enhancement of outflow facility is mediated through the trabecular meshwork CB1 receptor, with an involvement of p42/44 MAP kinase signaling pathway and changes in actin cytoskeletons. (*Invest Ophthalmol Vis Sci.* 2006;47:1999–2005) DOI:10.1167/iovs.05-0729

Cannabinoids are well known for their psychotropic properties, as well as for their therapeutic potential. The major targets of cannabinoid ligands are cannabinoid (CB)1 and CB2 receptors.[1,2] CB1 receptors are located in the central nervous system (CNS) as well as in the periphery, whereas CB2 receptors are mainly located in the peripheral tissues, such as immune cells.[1–3]

Glaucoma is one of the leading causes of blindness, and elevated intraocular pressure (IOP) is a major risk factor for glaucoma. The original report that marijuana smoking leads to a reduction in IOP was published more than three decades ago.[4] Since then, cannabinoids have been studied in humans and in animal models for their IOP-lowering effects and their potential as a new class of therapeutic agents for glaucoma.[5–11] In recent years, the involvement of cannabinoid receptors in controlling IOP has been explored.[8–11] It has been shown that CB1 receptors are at least partially responsible for the IOP-lowering effects of cannabinoids.[8,9]

Trabecular meshwork is a major site for aqueous humor outflow and thus is very important for regulating IOP. Recent studies have demonstrated the presence of CB1 receptor mRNAs and proteins in the trabecular meshwork cells.[12,13] However, currently it is unknown whether the trabecular meshwork cell CB1 receptors play a role in regulating aqueous humor outflow.

Noladin ether, the 2-glyceryl ether of arachidonyl ethanol, has been demonstrated to be a stable endogenous cannabinoid ligand selective for the CB1 receptor.[14] First of all, this study was performed to test whether noladin ether has a direct effect in modulating aqueous humor outflow. Second, the mechanism of noladin ether–induced enhancement of outflow facility was investigated: the existence of CB1 receptor proteins in trabecular meshwork cells, the signaling of these receptors through p42/44 MAP kinase, the involvement of CB1 receptors, and the involvement p42/44 MAP kinase signaling pathway in noladin ether–induced enhancement of outflow facility were studied. The possible involvement of matrix metalloproteinase (MMP)-2 and actin cytoskeleton in noladin ether–induced enhancement of outflow facility were examined.

Methods

Porcine Anterior Segment–Perfused Organ Culture Model

A previously published procedure[15] was used for the anterior segment–perfused organ culture model. Fresh porcine eyes were obtained from a local slaughterhouse within 30 minutes after decapitation. Porcine anterior segment explants, comprised the intact cornea, the undisturbed trabecular meshwork, and a 2- to 5-mm rim of sclera with the ciliary body and iris gently removed, were mounted in a standard

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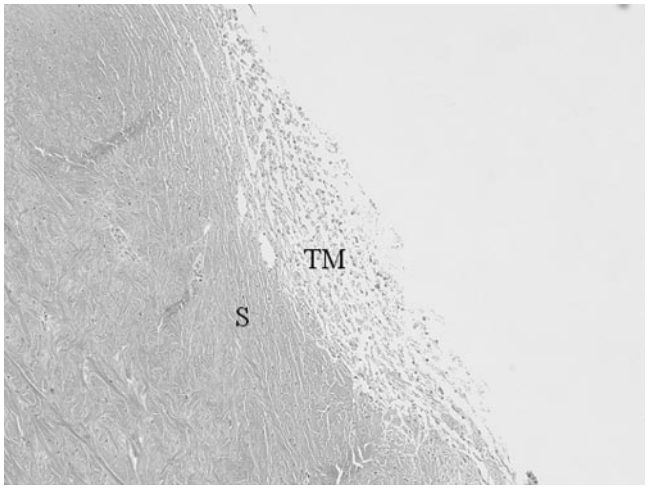

FIGURE 1. An example of intact trabecular meshwork staining. The anterior segment was processed and stained. TM, trabecular meshwork; S, Sclera.

perfusion culture apparatus and perfused with Dulbecco's modified Eagles medium (DMEM) using a constant perfusion head of 10 cm (~7.35 mm Hg) for 1 day, while outflow stabilized. 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 has been shown that in this model, outflow is through the trabecular meshwork, and flow rates are physiological (approximately 2.75 μL/min).[15] At the end of the perfusion study, the anterior segments were perfusion fixed at 7.35 mm Hg of 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. Perfusion studies were regarded as invalid and data discarded if more than one quadrant per eye had unacceptable morphologic findings, such as excessive trabecular meshwork cell loss and denudation of trabecular beams. An example of intact trabecular meshwork staining is shown in Figure 1.

After stabilization, noladin ether (Tocris Cookson, Inc., Ellisville, MO) was introduced by exchanging the perfusion chambers with drug-containing medium. The anterior segments were then perfused continuously with noladin-containing medium for 5 hours, and the outflow facility was monitored. Vehicle control was run in parallel. For the antagonist and inhibitor studies, the respective antagonist or inhibitor was applied to the perfusion medium 30 minutes before treatment with noladin and was present throughout the treatment. Outflow facility was calculated as the ratio of the rate of flow of perfusate (in microliters per minute) 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.

Ten eyes were used for each group of treatment. The data are presented as the mean ± SE and were analyzed on computer (Prism software; GraphPad, San Diego, CA) and plotted as change in outflow facility versus time (in minutes).

Culture of Porcine Trabecular Meshwork Cells

The trabecular meshwork was isolated from fresh porcine eyes by blunt dissection. Culture of trabecular meshwork cells was performed according to previously published methods.[16,17] The identity of trabecular meshwork cells was established by their morphology and their ability to take up acetylated low-density lipoprotein and to secrete tissue plasminogen activator.[16,17]

Immunofluorescence Microscopy

Cultured porcine trabecular meshwork cells were grown on coverslips (Fisher Scientific Inc., Pittsburgh, PA). Cells were washed twice with PBS, fixed with 4% paraformaldehyde for 15 minutes, and then washed twice again with PBS. Subsequently, cells were blocked for 1 hour at room temperature in PBS containing 5% normal goat serum (NGS), and then incubated for 2 hours at room temperature with anti-CB1 (Cayman Chemical, Ann Arbor, MI) or anti-paxillin (Upstate, Inc., Lake Placid, NY) antibody at 1:1000 dilution in PBS containing 5% NGS. After they were washed three times with PBS containing 5% NGS for 10 minutes each time, cells were incubated for 1 hour at room temperature with fluorescein isothiocyanate–conjugated goat anti-rabbit IgG secondary antibody (Zymed, South San Francisco, CA). Finally, the coverslips were washed four times with PBS, mounted with antifade medium (Vectashield; Vector Laboratories, Burlingame, CA), and viewed with a fluorescence microscope (model IX50; Olympus, Lake Success, NY).

Western Blot Analysis

Membrane samples were prepared from porcine trabecular meshwork cells according to published procedures.[18] Samples were incubated with 2× Laemmli buffer under reducing conditions 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. The nitrocellulose membranes were blocked with 5% nonfat dried milk in TBS-T buffer (10 mM Tris-HCl [pH 8.0] 150 mM NaCl, and 0.3% Tween 20) for 1 hour and then incubated overnight at 4°C with primary anti-CB1 or anti-MMP-2 antibody (Cell Signaling, Beverly, MA). Subsequently, the membranes were washed twice for 10 minutes each time with TBS-T buffer and incubated with horseradish peroxidase (HRP)–conjugated secondary antibody (GE Healthcare, Piscataway, NJ) 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 using an enhanced chemiluminescence (ECL) kit (GE Healthcare).

MAP Kinase Activity Assay

Before experiments, trabecular meshwork cells were maintained in serum-free medium overnight. To activate the p42/44 mitogen-activated protein (MAP) kinase, the cells were incubated with vehicle, noladin ether, noladin ether plus SR141716A, or SR141716A alone for 30 minutes. At the end of the incubation period, 200 μL of ice-cold lysis buffer containing 50 mM β-glycerophosphate, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM $NaVO_4$, 1 mM dithiothreitol (DTT), and 1 μg/mL of a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) were added. The cell lysates were incubated on ice for 5 minutes and then transferred to microcentrifuge tubes. The lysates were clarified by centrifugation at 10,000*g* for 10 minutes, the supernatants were collected, and protein concentrations were measured using the Bradford protein assay reagent (Bio-Rad, Hercules, CA). After boiling with 2× Laemmli sample buffer for 10 minutes, 40 μL of cell lysate (containing 25 μg of protein) was run on a 10% SDS-polyacrylamide gel. Subsequently, the proteins were transferred onto a nitrocellulose membrane, and the total p42/44 MAP kinase bands were detected by Western blot analysis by using a rabbit polyclonal anti-p42/44 MAP kinase antibody (Cell Signaling Technology, Beverly, MA). The levels of phosphorylated p42/44 MAP kinase were determined using a mouse monoclonal antibody against phospho-p44/42 MAP kinase (Thr202/Tyr204; Cell Signaling Technology, Inc. Beverly, MA), according to procedures described by the manufacturer.

Matrix MMP-2 Activity Assay

Trabecular meshwork cells were maintained in serum-free medium overnight before experiment. The cells were incubated with vehicle, noladin ether, noladin ether plus SR141716A, or SR141716A alone for

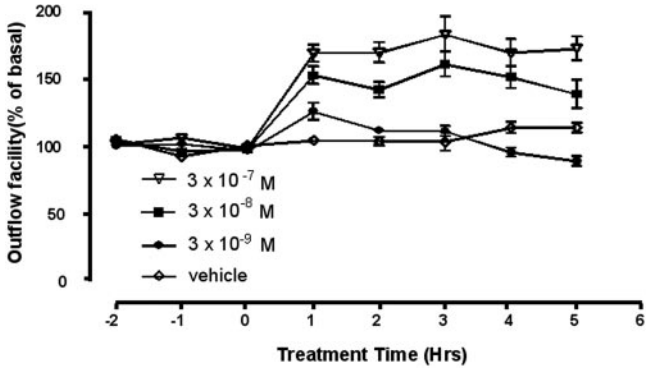

FIGURE 2. The effects of noladin ether on aqueous humor outflow facility. Three concentrations of noladin ether were used. Results are expressed as the mean ± SE; $n = 10$. The concentrations of 30 and 300 nM induced a significant difference in outflow facility when compared with that induced by vehicle.

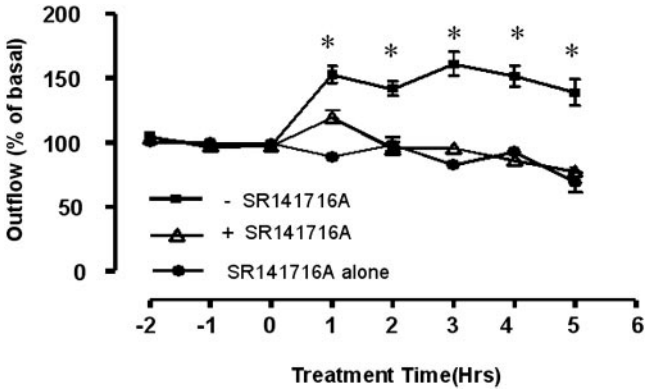

FIGURE 3. Antagonism of noladin ether–induced increase of aqueous humor outflow facility by SR141716A. The anterior segments were treated with 1 μM SR141716A for 30 minutes before the addition of 30 nM noladin ether. Results are expressed as the mean ± SE; $n = 10$. *Significant differences between noladin ether alone and noladin ether plus SR141716A ($P < 0.05$, *t*-test).

2 hours, after which the medium was collected and concentrated (Centricon concentrator; Millipore Inc., Bedford, MA). The concentrated samples were mixed with equal volume of 2× sample buffer (125 mM Tris-HCl [pH 6.8] 4% SDS, 20% glycerol, 0.01% bromphenol blue). Samples were then run on a SDS-polyacrylamide gel containing 0.1% gelatin. Gel was washed twice with 2.5% Triton X-100 for 30 minutes each time and incubated overnight at room temperature with incubation buffer (50 mM Tris-HCl [pH 7.5], 5 mM CaCl, 1% Triton X-100, 200 mM NaCl, 0.05% NaN_3). Subsequently, the gel was stained with Coomassie blue for 1 hour and destained in methanol/acetic acid (10%/10%).

Phalloidin Staining of Actin Cytoskeleton

For phalloidin staining of actin cytoskeleton, trabecular meshwork cells were plated, treated, fixed and permeabilized as in immunofluorescence microscopy section. Subsequently, the coverslips were blocked with 3% bovine serum albumin in PBS for 10 minutes. After a brief washing with PBS, AlexaFluor 488-conjugated phalloidin (0.67 units/mL; Invitrogen) was added to stain F-actin. Slides were then mounted and viewed by immunofluorescence microscopy.

Data Analyses

The bands on x-ray films or zymography gels were scanned (Personal Densitometer SI; Molecular Dynamics, Sunnyvale, CA) and were semiquantified (ImageQuant; Molecular Dynamics).

For anterior segment perfusion studies, unpaired two-tailed Student's *t*-tests were used to compare the data points of the treatment groups. For p42/44 MAP kinase assays, ANOVA with Newman-Keuls posttests were used. The level of significance was chosen as $P < 0.05$.

RESULTS

The Effects of Noladin Ether on Outflow Facility

Outflow facility studies were performed using the porcine anterior segment perfused organ culture model. As shown in Figure 2, the outflow facility was increased concentration-dependently within 1 hour after adding 3, 30, and 300 nM of noladin ether, an endogenous cannabinoid agonist selective for the CB1 receptor. This noladin ether–induced enhancement of outflow facility lasted for 5 hours with 30- and 300-nM concentrations, showing significant differences from vehicle control. In addition, the effect of 30 nM noladin ether was completely blocked by pretreatment of anterior segments with 1 μM of SR141716A,[19] a highly selective CB1 antagonist (Fig. 3). In control experiments, either DMEM or SR141716A alone was added. Neither the medium (Fig. 2) nor SR141716A (Fig. 3) alone had any significant effects on outflow facility.

Identification of CB1 Receptors on Trabecular Meshwork Cells

Immunofluorescence microscopy and Western blot analysis were performed to determine whether CB1 receptors are expressed on trabecular meshwork cells. In immunofluorescence microscopy studies, positive signals were detected on porcine trabecular meshwork cells with a primary anti-CB1 antibody (Fig. 4A). In contrast, when the primary anti-CB1 antibody was preabsorbed with peptide antigen, only weak, nonspecific background signals were detected (Fig. 4B). In Western blot analysis, a protein band with a molecular mass of approximately 64 kDa was detected with the anti-CB1 antibody, but not with the same antibody preabsorbed with peptide antigen (Fig. 4C).

The Effects of Noladin Ether on p42/44 MAP Kinase Phosphorylation in Trabecular Meshwork Cells

One of the signaling pathways for CB1 receptor is stimulation of p42/44 MAP kinase. To explore whether the CB1 receptors

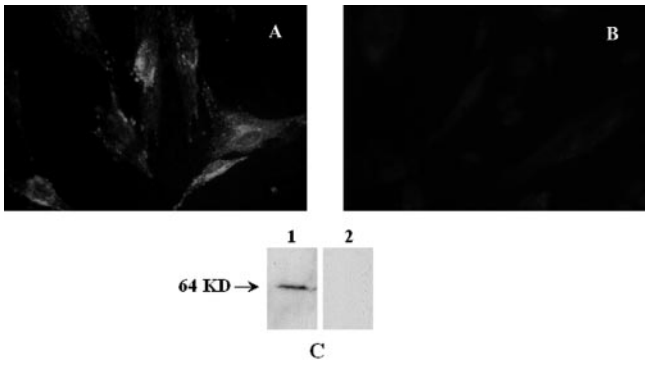

FIGURE 4. Expression of CB1 receptors on porcine trabecular meshwork cells. (**A**) CB1 receptor immunofluorescence observed with a primary anti-CB1 antibody. (**B**) Background immunofluorescence observed with the primary anti-CB1 antibody preabsorbed with the peptide antigen. (**C**) Western blot analysis: *Lane 1*: primary anti-CB1 antibody; *lane 2*: primary anti-CB1 antibody preabsorbed with the peptide antigen.

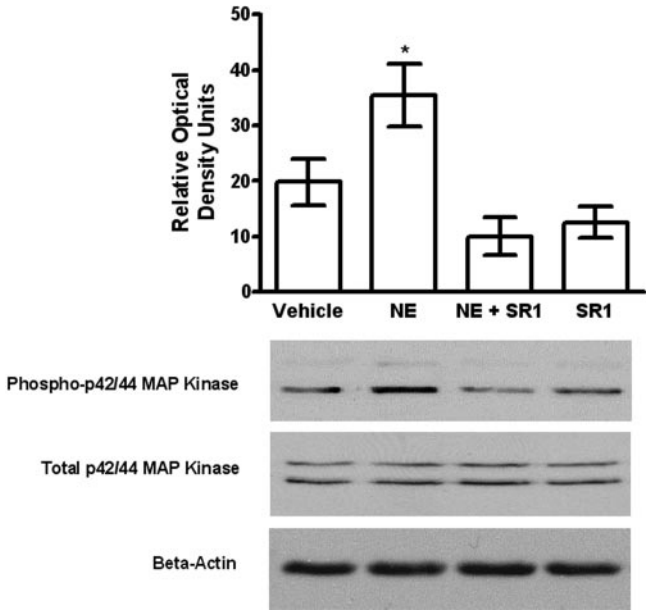

FIGURE 5. The effects of noladin ether on p42/44 MAP kinase activity in trabecular meshwork cells. *Top*: densitometry quantification of the phospho-p42/44 data from five experiments. *Significant difference from vehicle alone ($P < 0.05$ ANOVA with Newman-Keuls posttest). The concentrations of noladin ether and SR141716A were 30 nM and 1 μM, respectively. Protein (25 μg) was loaded in each lane. The time of noladin ether stimulation was 30 minutes. *Bottom*: gel representative of results obtained in five experiments.

expressed on trabecular meshwork cells are functionally coupled to this signaling pathway, we studied the effect of noladin ether on p42/44 MAP kinase activity of trabecular meshwork cells. As shown in Figure 5, treatment with 30 nM noladin ether for 30 minutes resulted in an increased phosphorylation of p42/44 MAP kinase compared with vehicle treatment. The total p42/44 MAP kinase level was not altered by noladin ether treatment. Pretreatment of trabecular meshwork cells for 30 minutes with 1 μM SR141716A, a selective CB1 antagonist, had no significant effect by itself, but blocked noladin ether–induced p42/44 MAP kinase phosphorylation.

Effects of PD98059 on Noladin Ether–Induced Enhancement of Outflow Facility

To correlate the data of noladin ether on outflow facility with our findings of noladin ether on p42/44 MAP kinase activity, PD98059, an inhibitor of the p42/44 MAP kinase pathway, was used to explore the possibility that p42/44 MAP kinase may be involved in the noladin ether–induced enhancement of outflow facility. As shown in Figure 6, pretreatment of the perfused porcine anterior segments with 30 μM of PD98059 significantly blocked the outflow-enhancing effects of 30 nM noladin ether. PD98059 by itself had no significant effect on outflow facility.

Effects of Noladin Ether on Matrix MMP-2

MMP-2 is a member of zinc-dependent enzyme secreted by trabecular meshwork cells. MMP-2 regulates extracellular matrix, which is critical for controlling movement of aqueous humor.[20–22] To investigate whether MMP-2 plays a role in noladin ether–induced enhancement of aqueous humor outflow, trabecular meshwork cells were incubated with vehicle, noladin ether, noladin ether plus SR141716A, or SR141716A alone for 3 hours. The amount of MMP-2 secreted and the MMP-2 activity were monitored by Western blot analysis and zymography, respectively. Our data indicate that neither MMP-2 secretion nor MMP-2 activity was significantly affected by noladin ether treatment (data not shown).

Effects of Noladin Ether on Actin Cytoskeleton

As illustrated in Figure 7A, control trabecular meshwork cells showed polygonal morphology; cells appeared well spread; the elongated actin stress fibers were prominently visible. By contrast, noladin ether treatment led to changes in trabecular meshwork cell morphology and actin cytoskeleton; the cells appear rounded up and less spread out, with many filipodia-like structures; the actin was present in the form of shorter stress fibers and appeared to form fine phalloidin-stained structures throughout the periphery of the cells (Fig. 7B). Both SR141716A, a CB1 antagonist, and PD98059, an inhibitor of the p42/44 MAP kinase pathway, were able to block the effects of noladin ether—that is, the cells treated with noladin ether plus SR141716A or noladin ether plus PD98059 exhibited most of the morphologic features associated with control cells (Figs. 7C, 7E). Trabecular meshwork cells treated with SR141716A or PD98059 by itself also exhibited morphology and actin stress fibers similar to the untreated cells (Figs. 7D, 7F).

As shown in Figure 8A, immunofluorescence microscopy with an anti-paxillin antibody demonstrated abundant focal adhesion structures on control trabecular meshwork cells. Treatment of cells with noladin ether caused a decrease in the number of focal adhesion structures (Fig. 8B). This modulatory effect of noladin ether on focal adhesion was blocked by SR141716A, a selective CB1 antagonist and PD98059, an inhibitor of the p42/44 MAP kinase pathway (Figs. 8C, 8E), whereas SR141716A or PD98059 by itself had no effects on trabecular meshwork cell focal adhesion structures (Figs. 8D, 8F).

DISCUSSION

IOP is regulated by the delicate balance between the rate of aqueous humor secretion from the ciliary epithelium and its outflow through the trabecular meshwork and uveoscleral route. The localizations of cannabinoid receptors in trabecular meshwork suggest the involvement of these receptors in regulating outflow facility.[11–13] In a recent study, we demonstrated the presence of CB2 receptors in trabecular meshwork and the involvement of CB2 receptors in the enhancement of outflow facility induced by JWH015, a selective CB2 agonist.[11] However, until the present study the involvement of CB1 receptors in regulating aqueous humor outflow has yet to be established. Noladin ether

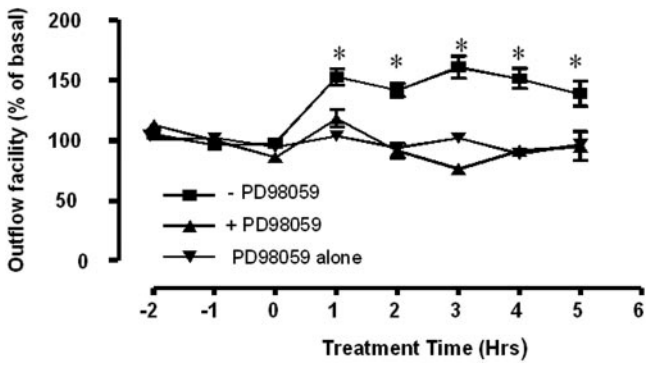

FIGURE 6. The effects of pretreatment with PD98059 on noladin ether–induced enhancement of aqueous humor outflow facility. The anterior segments were treated with 30 μM of PD98059 30 minutes before the addition of 30 nM noladin ether. Results are expressed as the mean ± SE; $n = 10$. *Significant difference between the noladin ether alone and noladin ether plus PD98059 ($P < 0.05$, *t*-test).

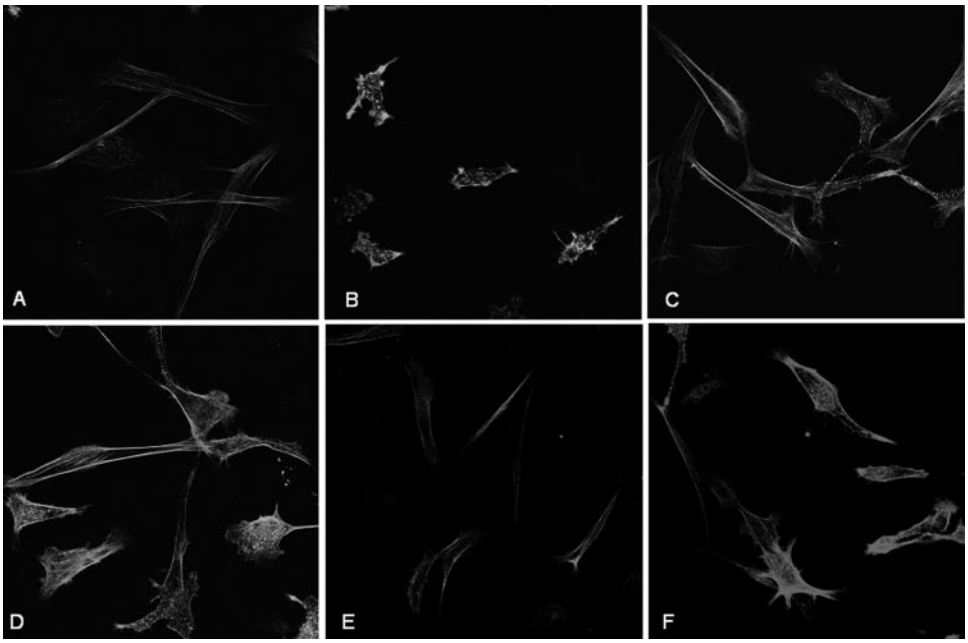

FIGURE 7. Effects of noladin ether on trabecular meshwork cell morphology and actin cytoskeleton. Trabecular meshwork cells were plated on fibronectin-coated coverslips for 18 hours and then treated for 3 hours with vehicle (**A**), 30 nM noladin ether (**B**), 30 nM noladin ether plus 1 μM SR141716A (**C**), 1 μM SR141716A (**D**), 30 nM noladin ether plus 30 μM PD98059 (**E**), or 30 μM PD98059 (**F**). The trabecular meshwork cells were then fixed and stained with AlexaFluor 488-labeled phalloidin.

is an endogenous cannabinoid agonist that is selective for the CB1 receptor.[14] The IOP-lowering effects of noladin ether have been reported.[23] However, until the present study, it is unknown whether noladin ether directly acts on trabecular meshwork to increase aqueous humor outflow.

The present study used perfused anterior segments of the porcine eye to examine the regulation of aqueous outflow by noladin ether. This is an excellent model for studying aqueous humor outflow as it preserves the critical architecture of the outflow pathway and is not affected by biological changes outside the eye.[24,25] In this study, application of noladin ether enhanced aqueous humor outflow facility. This outflow-enhancing effect of noladin ether was concentration-dependent, and was blocked by pretreatment with SR141716A, a selective CB1 antagonist (Figs. 2, 3). Thus, the data from the current anterior segment perfusion study indicate an involvement of CB1 receptors in noladin ether–induced increase in outflow facility.

By the use of affinity-purified polyclonal antibodies against CB1 receptor protein, Straiker et al.[12] first demonstrated the distribution of CB1 receptor proteins in different regions of the human eye, including the trabecular meshwork. Subsequently, Stamer et al.[13] reported the presence of CB1 receptor proteins in bovine as well as human trabecular meshwork, in a study using immunofluorescence microscopy. In the present study, using both immunofluorescence microscopy and Western blot analysis, we detected CB1 receptor proteins on porcine trabecular meshwork cells with an anti-CB1 primary antibody (Fig. 4). Our current results clearly confirm the findings of Staiker et al. and Stamer et al.

Although studies have shown that CB1 receptors in trabecular meshwork are involved in the activation of G-proteins and

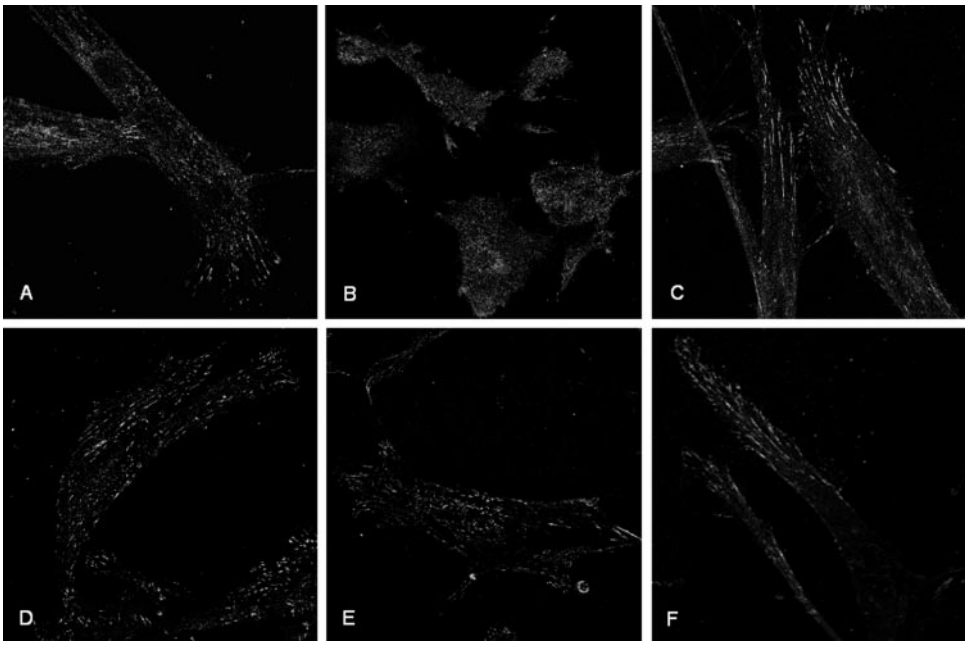

FIGURE 8. Effects of noladin ether on trabecular meshwork cell focal adhesion structures detected with an anti-paxillin antibody. Trabecular meshwork cells were plated on fibronectin-coated coverslips for 18 hours then treated for 3 hours with vehicle (**A**), 30 nM of noladin ether (**B**), 30 nM of noladin ether plus 1 μM SR141716A (**C**), 1 μM of SR141716A (**D**), 30 nM of noladin ether plus 30 μM of PD98059 (**E**), or 30 μM of PD98059 (**F**). The trabecular meshwork cells were then fixed and stained with an anti-paxillin antibody.

stimulation of cAMP,[13] the coupling of trabecular meshwork CB1 receptors to p42/44 MAP kinase, another well-recognized signaling pathway for the CB1 receptors,[26] has not been reported. In the present study, the highly selective endogenous CB1 agonist noladin ether was shown to activate p42/44 MAP kinase pathway in cultured porcine trabecular meshwork cells. This effect of noladin ether was blocked by pretreatment of the cells with SR141716A, a selective CB1 antagonist (Fig. 5). These data demonstrate the existence of functional trabecular meshwork CB1 receptors that are coupled to the p42/44 MAP kinase pathway.

Our data on the noladin ether–induced activation of p42/44 MAP kinase in the trabecular meshwork, together with previous findings that p42/44 MAP kinase plays an important role in the cellular functions of trabecular meshwork cells,[27,28] prompted us to hypothesize that noladin ether–induced increases of aqueous humor outflow may be mediated through the p42/44 MAP kinase pathway. This hypothesis was further supported by our experimental results that pretreatment of the perfused anterior segments with PD90859, an inhibitor of the p42/44 MAP kinase pathway, blocked noladin ether–induced enhancement of outflow facility (Fig. 6).

In this study, two possible cellular mechanisms by which noladin ether may exert its aqueous-outflow– enhancing effects were explored. Experiments were performed to test the hypotheses that noladin ether may change the secretion/activity of MMP-2 and/or the actin cytoskeleton of trabecular meshwork cells.

Although studies have shown that MMP-2 plays an important role in modulating aqueous outflow,[20–22] our results indicate that neither MPP-2 secretion nor its activity was changed significantly when trabecular meshwork cells were stimulated by noladin ether. These data suggest that noladin-induced enhancement of aqueous humor outflow is not through the MMP-2 pathway.

In contrast, treatment with noladin ether resulted in changes in trabecular meshwork cell morphology, actin cytoskeleton, and focal adhesion structures (Figs. 7, 8). These effects of noladin ether were blocked by SR141716A, a selective CB1 antagonist and PD98059, an inhibitor of the p42/44 MAP kinase pathway. These data on trabecular meshwork cells are consistent with a previous report of ours on the CB1 receptor-mediated morphologic and actin cytoskeleton changes in a neuronal cell line.[29] Previous studies have demonstrated that trabecular meshwork cell cytoskeleton is involved in the regulation IOP and several cytoskeleton-modulating drugs enhance aqueous humor outflow.[30,31] Therefore, it is not unreasonable to infer from our current data that in vivo, noladin ether may act on CB1 receptors to increase aqueous humor outflow through a mechanism involving trabecular meshwork cell morphology and actin cytoskeleton changes.

In conclusion, the present study reports for the first time that administration of noladin ether, an endocannabinoid agonist selective for the CB1 receptor, increases aqueous humor outflow facility. The noladin ether–induced enhancement of outflow facility is mediated through the trabecular meshwork CB1 receptors, with an involvement of p42/44 MAP kinase signaling pathway. Furthermore, noladin ether may alter outflow facility through changes of trabecular meshwork cytoskeleton.

There are already plenty of drugs on the market to achieve the suppression of aqueous humor formation. In contrast, there are few drugs on the market that target the conventional aqueous humor outflow. Because CB1 and CB2 receptors have been found to be involved in regulating aqueous humor outflow facility, cannabinoid compounds targeted at these receptors may be useful additions to the therapeutic agents for glaucoma treatment.

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