

First investigations of endocannabinoid function in human ocular cells using cellular dielectric spectroscopy

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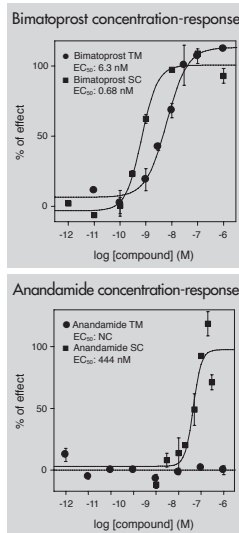
INTRODUCTION

- Cannabis and the endocannabinoids have long been known to reduce intraocular pressure. Thus, in the glaucomatous monkey model, anandamide, abn-cannabidiol both lower intraocular pressure. Potent and selective Fatty acid amide hydrolase inhibitors do not affect intraocular pressure, thereby suggesting no control of aqueous humor dynamics by endogenous endocannabinoid.
- The aim of this study was to investigate the molecular mechanism by which the anandamide reduces intraocular pressure. For this purpose, we examined the effect of endocannabinoids on two human ocular cell types of the aqueous humor outflow pathway known to control intraocular pressure: cells from the trabecular meshwork (TM cells) and endothelial cells of Schlemm's canal (SC cells). Using cellular dielectric spectroscopy (CellKey™ technology), we measured the activity of anandamide on both cell types and elucidated its target and downstream signalling pathway.

Anandamide response in TM and SC cells

Bimatoprost ► was used as a positive control on TM cells and SC cells. As we have previously shown¹, Bimatoprost specifically activates the prostamide F2 α receptor in both cell types of the human conventional outflow pathway to modify intraocular pressure.

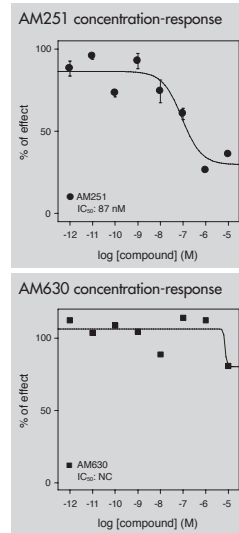
Anandamide ► induces a concentration-response in SC cells but not in TM cells.



Anandamide target in SC cells

The CB₁ receptor selective antagonist AM251 inhibits the anandamide response in SC cells, whether the CB₂ selective antagonist AM630 does not.

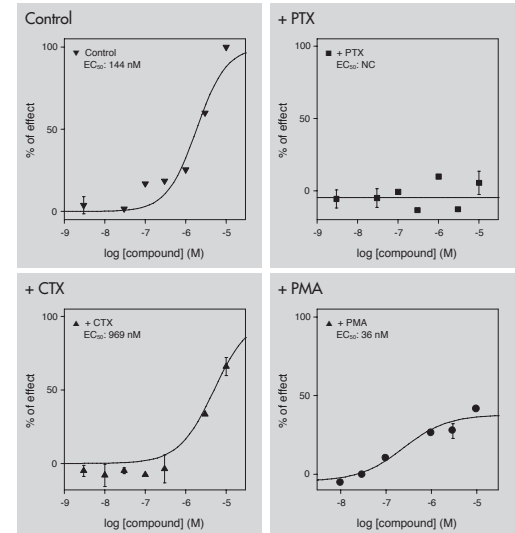
This result suggests the involvement of the CB₁ receptor in the effect of the endocannabinoid.



Anandamide signalling pathway in SC cells

An overnight treatment of cells with PMA (which inhibits the PKC) or CTX (which inhibits the Gs protein signalling pathway) does not block the anandamide concentration-response.

However, an overnight treatment of cells with PTX (which inhibits the Gi protein signalling pathway) blocks the anandamide concentration-response, confirming the involvement of the Gi protein signalling pathway.



¹ Stamer, W.D. et al. (2010) Invest. Ophthalmol. Vis. Sci.; 0: i-ivs. 09-4955v1-i-ivs. 09-4955.

Material & methods

■ **CellKey™ experiment:** For these studies, cells were seeded onto the CellKey™ standard 96W microplate and allowed to grow in growth medium under standard tissue culture conditions. Growth medium was changed to Hanks-Hepes buffer supplemented with 0.1% BSA 1 hour before the start of the experiment. Plates were placed onto the CellKey™ system and pre-addition measurements were made for 5 minutes to obtain a baseline reading. Then, ligands were added simultaneously to all 96 wells using an integrated fluidics system. Impedance measurements were made for 10 minutes after ligand addition to monitor cellular responses to ligand interaction. CellKey™ measurements in this study were collected at 37°C. For the experiment with the antagonists, ligands were pre-incubated with cells for 15 minutes before the addition of the anandamide at 10 μ M. Cholera toxin (20 μ g/mL), pertussis toxin (10 ng/mL) and PMA (100 nM) were pre-incubated with cells overnight before the addition of the anandamide at 10 μ M. The specific time was calculated by subtracting the background signal (Buffer only) from the total signal at 10 minutes.

■ **Cell culture:** Enucleated human donor eyes and eye tissues were obtained from the Life Legacy Foundation (Tucson, AZ), National Disease Research Interchange (Philadelphia, PA) and Sun Health Research Institute (Sun City, AZ). Schlemm's canal (SC) cells were isolated from conventional outflow tissues of whole human eyes or post-penetrating keratoplasty surgical remnants using a cannulation technique and then were characterized and cultured. Trabecular meshwork (TM) cells were isolated from human eyes using a blunt dissection procedure followed by extracellular matrix digestion and were characterized and cultured. Cell isolates were maintained in Dulbecco's modified Eagle's medium (DMEM, low glucose), supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (0.1 mg/mL) and glutamine (0.29 mg/mL).

CONCLUSION

- Anandamide may be inactive on cells from the trabecular meshwork but was active on endothelial cells of Schlemm's canal, indicating that the endocannabinoids may control aqueous humor dynamics at the level of Schlemm's canal.
- Experiments using selective antagonists and signalling pathway modulators suggest that the anandamide acts on endothelial cells of Schlemm's canal via the CB₁ receptor and its downstream Gi protein signalling pathway.

Acknowledgements

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