Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 330 (2005) 1062–1067
BBRC
www.elsevier.com/locate/ybbrc

Finding of endocannabinoids in human eye tissues: Implications for glaucoma ☆

June Chen [a,*], Isabel Matias [b], Tim Dinh [a], Ta Lu [a], Sonia Venezia [b], Amelia Nieves [a], David F. Woodward [a], Vincenzo Di Marzo [b,*]

[a] *Department of Biological Sciences, Allergan Inc., Irvine, CA, USA*
[b] *Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli (Napoli), Italy*

Received 9 March 2005
Available online 23 March 2005

Abstract

Cannabinoid CB_1 receptors are involved in ocular physiology and may regulate intraocular pressure (IOP). However, endocannabinoid levels in human ocular tissues of cornea, iris, ciliary body, retina, and choroid from normal and glaucomatous donors have not been investigated. Anandamide (*N*-arachidonoylethanolamine; AEA), 2-arachidonoylglycerol (2-AG), and the anandamide congener, palmitoylethanolamide (PEA), were detected in all the human tissues examined. In eyes from patients with glaucoma, significantly decreased 2-AG and PEA levels were detected in the ciliary body, an important tissue in the regulation of IOP. The findings suggest that these endogenous compounds may have a role in this disease, particularly with respect to regulation of IOP.
© 2005 Elsevier Inc. All rights reserved.

Keywords: 2-Arachidonoylglycerol; Anandamide; Palmitoylethanolamide; Eye; Human; Glaucoma

Endocannabinoids are endogenous substances that activate cannabinoid (CB_1 and CB_2) receptors and other molecular targets, including non-CB_1, non-CB_2 G-protein-coupled receptors, and various ion channels [1–3]. The two most studied endocannabinoids are 2-arachidonoylglycerol (2-AG) and anandamide (*N*-arachidonoylethanolamine, AEA). 2-AG is described as a physiologically essential molecule that is the most specific and abundant endogenous agonist at CB_1 and CB_2 receptors [3,4]. AEA has a greater selectivity for CB_1 than CB_2 receptors, behaves as a partial agonist at cannabinoid receptors in several systems, and activates also vanilloid type 1 (TRPV1; transient receptor potential vanilloid type 1) receptors [1,2,5,6]. Palmitoylethanolamide (PEA) is an endogenous congener of AEA that does not activate CB_1 or CB_2 receptors [2,7]. PEA is co-synthesized with AEA by most cell types and was proposed to enhance AEA effects mediated by both CB_1 and TRPV1 receptors [1,7–9]. Endocannabinoids, enzymes responsible for their degradation, notably fatty acid amide hydrolase (FAAH), and biosynthesis, and cannabinoid receptors comprise the endocannabinoid system [4,10]. Although 2-AG, AEA, and PEA have been identified in ocular tissues of various species, investigations in human eyes were restricted to quantification of AEA in non-glaucomatous trabecular meshwork, ciliary process, and neurosensory retinal tissues [11].

☆ *Abbreviations:* AEA, anandamide, *N*-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; CB, cannabinoid; FAAH, fatty acid amide hydrolase; IOP, intraocular pressure; LC-APCI-MS, liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D; MAGL; monoacylglycerol lipase; PEA, palmitoylethanolamide; POAG, primary open-angle glaucoma; PLC, phospholipase C; PLA_2, phospholipase A_2; TRPV1, transient receptor potential vanilloid type 1.

* Corresponding authors. Fax: +1 714 246 5578 (J. Chen), +39 081 8041770 (V. Di Marzo).
E-mail addresses: chen_june@allergan.com (J. Chen), vdimarzo@icmib.na.cnr.it (V. Di Marzo).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.095

The function of endocannabinoids in the eye encompasses the regulation of photoreception and neurotransmission in the optic nerve [12–15], and includes a protective action in an experimental allergic uveitis model, through actions that are mostly mediated by CB_1 receptors [16]. Studies show that topical administration of synthetic CB_1 agonists lowers intraocular pressure (IOP) of rabbits, non-human primates, and glaucomatous humans [10,17–19]. The mechanism of ocular hypotension by CB_1 agonists in cynomolgus monkeys appears to involve a decrease in aqueous humor flow [19]. Endocannabinoids may also provide tonic regulation of IOP as shown by the association of ocular hypotension with increases in endocannabinoid levels, produced by inhibiting their inactivation by FAAH or cellular reuptake [20,21].

The presence of a functional endocannabinoid system in the eye supports a role for endocannabinoids in ocular physiology. However, endocannabinoid levels in different eye tissues from donors exhibiting normal or a pathological condition such as glaucoma have never been investigated. The principal aim of the present study was to determine the levels of 2-AG, AEA, and PEA in human ocular tissues (cornea, iris, ciliary body, retina, and choroid) from normal and glaucomatous donors.

Materials and methods

Human eyes. Normal eyes ($n = 12$) or glaucomatous eyes ($n = 12$) of donors were obtained from the National Disease Research Interchange (Philadelphia, PA, USA) and the Central Florida Lions Eye and Tissue Bank (Tampa, FL, USA). The organ banks obtained informed consent from all donors or their family members. The mean donor age was 70.5 ± 2.6 years (range 49–90) for normal eyes and 76.0 ± 3.5 years (range 56–91) for glaucomatous eyes. The eyes were enucleated 2–10 h after death, stored in phosphate-buffered saline in the refrigerator (4 °C), and delivered between 1 and 4 days following death. Upon receipt, the eyes were stored frozen at −80 °C until use.

Endocannabinoid extraction. The cadaveric eyes were thawed overnight in the refrigerator (4 °C) for each assay. The cornea, iris, ciliary body, retina, and choroid were quickly dissected and weighed. Tissues were immediately placed into Eppendorf tubes containing 200 μl freshly prepared solution of chloroform/methanol (2:1 by volume) and 100 μl Tris–HCl 50 mM, pH 7.4. Samples were cooled on ice for the homogenization and extraction processes. The cornea and iris were minced and all tissues were homogenized using a pestle. An additional 300 μl of the chloroform/methanol/Tris–HCl solution was added to rinse the pestle. Samples were vortex-mixed and sonicated (3 min) in a water bath. Endocannabinoids were extracted from the aqueous phase by adding 600 μl chloroform to the samples, followed by vortex-mixing (1 min), centrifugation at 5030*g* (1 min) to separate the aqueous and organic phases, and the organic phase was transferred to silanized glass vials; this procedure was repeated three more times. The extracts were concentrated to dryness under nitrogen and weighed. The oxygen in the vial was eliminated using nitrogen and vials were capped, sealed with parafilm, and stored in the freezer at −20 °C until processing for the determination of endocannabinoid content. Deuterated compounds (d_5-2-AG and d_8-AEA; Cayman Chemical, Ann Arbor, MI, USA, and d_4-PEA, synthesized at the Institute of Biomolecular Chemistry, Pozzuoli, Italy) at 100 pmol (in 10 μl ethanol or methanol) each were added as internal standards.

Purification and quantification of 2-AG, AEA, and PEA. Lipid-containing organic phase was pre-purified by open-bed chromatography on silica gel mini-columns. The separated lipids (9:1 fraction) were analyzed by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2010) quadrupole MS via a Shimadzu APCI interface. MS analyses were carried out in the selected ion monitoring (SIM) mode as described previously [22]. Temperature of the APCI source was 350 °C; the HPLC column was a Phenomenex (5 μm, 150×4.5 mm) reverse-phase column, eluted with a mobile phase of methanol/water/acetic acid 85:15:0.2 (v/v/v) at a flow rate of 1 ml/min. AEA (retention time: 14.5 min, $m/z = 348.3$ for the native compound and 356.3 for the deuterated standard) and 2-AG (retention time: 17.0 min, $m/z = 379.0$ for the native compound and 384.0 for the deuterated standard) quasi-molecular ions were quantitated by isotope dilution with the deuterated standards and their amounts in picomoles were normalized per gram of wet tissue weight. PEA was quantified by comparison with its external synthetic standard, run under the same conditions (retention time: 19.0 min, $m/z = 300.3$ for the native compound and 304.3 for the deuterated standard).

Statistical analysis. The concentrations of endocannabinoids in tissues were expressed as picomoles (pmols) per gram (g) of wet tissue and presented as mean ± SEM. Statistical comparisons between normal and glaucomatous eye tissues consisted of testing for significance of difference between means using Student's *t* test for unpaired samples. Differences are considered statistically significant if the *P* value is $\leqslant 0.05$.

Results

Comparison of endocannabinoid levels in the different eye tissues

In human normal and glaucomatous eyes, 2-AG, PEA, and AEA were detected in all the tissues examined. The levels of individual substances varied between the ocular tissues and ranged as follows: 2-AG (range 62–1393 pmol/g tissue; Fig. 1), PEA (range 95–637 pmol/g tissue; Fig. 2), and AEA (range 24–178 pmol/g tissue;

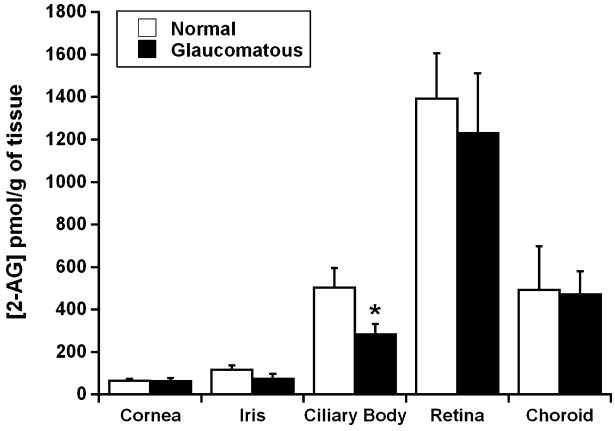

Fig. 1. 2-Arachidonoylglycerol levels (pmol/g tissue) in human eye tissues. Results are expressed as means ± SEM of 9–11 tissues. *Significantly different between normal and glaucomatous tissues ($P \leqslant 0.05$).

Download English Version:

https://daneshyari.com/en/article/9884425

Download Persian Version:

https://daneshyari.com/article/9884425

Daneshyari.com