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The Endocannabinoid System Is Present in Rod Outer Segments from Retina and Is Modulated by Light

Estefanía Chamorro Aguirre[1] · Virginia L. Gaveglio[1,2] · Susana J. Pasquaré[1,2]

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Abstract
The aim of the present research was to evaluate if the endocannabinoid system (enzymes and receptors) could be modulated by light in rod outer segment (ROS) from bovine retina. First, we analyzed endocannabinoid 2-arachidonoylglycerol (2-AG) metabolism in purified ROS obtained from dark-adapted (DROS) or light-adapted (LROS) retinas. To this end, diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), and lysophosphatidate phosphohydrolase (LPAP) enzymatic activities were analyzed using radioactive substrates. The protein content of these enzymes and of the receptors to which cannabinoids bind was determined by immunoblotting under light stimulus. Our results indicate that whereas DAGL and MAGL activities were stimulated in retinas exposed to light, no changes were observed in LPAP activity. Interestingly, the protein content of the main enzymes involved in 2-AG metabolism, phospholipase C β_1 (PLCβ_1), and DAGLα (synthesis), and MAGL (hydrolysis), was also modified by light. PLCβ_1 content was increased, while that of lipases was decreased. On the other hand, light produced an increase in the cannabinoid receptors CB1 and CB2 and a decrease in GPR55 protein levels. Taken together, our results indicate that the endocannabinoid system (enzymes and receptors) depends on the illumination state of the retina, suggesting that proteins related to phototransduction phenomena could be involved in the effects observed.

Keywords Endocannabinoid system · Diacylglycerol lipase · Lysophosphatidate phosphatase · Monoacylglycerol lipase · Rod outer segment · Phototransduction

Abbreviations

ABHD6	Serine hydrolase a/b-hydrolase domain-containing 6
2-AG	2-Arachidonoylglycerol
AEA	Anandamide
BROS	Rod outer segments obtained in darkness and subsequently illuminated
BSA	Bovine serum albumin; CB1, cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CNS	Central nervous system
DAG	Diacylglycerol
DAGL	Diacylglycerol lipase
DTT	Dithiothreitol
DROS	Rod outer segments obtained from dark-adapted retinas
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
GPR55	G protein–coupled receptor 55
HEPES	N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]
LPA	Lysophosphatidic acid
LPAP	Lysophosphatidate phosphohydrolase
LROS	Rod outer segments obtained from bleached retinas
MAG	Monoacylglycerol
MAGL	Monoacylglycerol lipase
NAPE-PLD	N-arachidonoyl-phosphatidylethanolamine phospholipase D
NEM	N-Ethylmaleimide
RIS	Rod inner segments
ROS	Rod outer segments
TLC	Thin-layer chromatography

✉ Susana J. Pasquaré
pasquare@criba.edu.ar

[1] Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB, UNS-CONICET), Edificio E1, Camino La Carrindanga km 7, 8000 Bahía Blanca, Argentina

[2] Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina

TRPV1	Transient receptor potential cation channel

Introduction

The retina is an integral part of the central nervous system (CNS) that communicates directly with the brain through the optic nerve. It is composed of six types of neuronal cells, namely photoreceptor, horizontal, bipolar, amacrine, interplexiform, and ganglionar. It also contains glial cells which are called Müller cells and which surround neuronal cells. The vertebrate photoreceptor cells are neurosensitive cells with unique morphology and a specialized function. They respond to the stimulus of light and transmit this response to adjacent neurons to finally send it to the visual centers of the brain. There are two types of photoreceptors: rods that are responsible for night vision (scotopic vision) and cones that mediate color vision and operate at high light intensities (photopic vision). Rods cells are highly specialized neurons which are characterized by the presence of three regions: (1) an outer segment (ROS), formed by membranous disks surrounded by a plasma membrane that contains the visual pigment rhodopsin and the proteins involved in phototransduction, (2) an inner segment (RIS) rich in organelles responsible for synthesizing the molecules that make up the disk membrane [1], and (3) a synaptic terminal from which the neurotransmitter glutamate is released to the adjacent neuron. The transduction of the light signal in ROS of the vertebrate photoreceptors is mediated by a series of reactions among different proteins that form protein-protein complexes either within or on the surface of the disk and in the plasma membrane [2]. Results from different research groups have shown that the constitutive phospholipids of these membranes are metabolically active and that they, like the enzymes responsible for their metabolism, are involved in the generation of physiological mediators in close relation with the transduction of the visual stimulus [3].

The endocannabinoid system (ECS), a newly known cell communication mechanism, basically consists of three elements: (1) the endogenous ligands anandamide (AEA) [4] and 2-arachidonoylglyceol (2-AG) [5], (2) the canonical membrane receptors CB1 and CB2 [6], and (3) the mechanisms for AEA synthesis (N-arachidonoyl-PE PLD (NAPE-PLD)) [7] and inactivation (FAAH) [8], and for 2-AG synthesis (PLC/DAGL [9, 10]and lysophosphatidate phosphohydrolase (LPAP) [11]) and inactivation (MAGL [12], FAAH [13], and ABHD [14]).

Numerous evidences support the presence of the ECS in a great variety of organs that fulfill different functions [5]. In spite of the vast literature on the subject, little is known about the role of this signaling system in the retinal function and more precisely in the retinal site where phototransduction takes place. In this respect, it has been reported that the retina is equipped with a functional ECS, which consists of endogenous cannabinoids, some of the enzymes that participate in its metabolism and the CB1 [15, 16] and CB2 receptors [17]. Interestingly, it has also been demonstrated that GPR55, another protein G–coupled receptor that interacts with cannabinoids, co-localizes with rhodopsin in ROS from primate retina [18, 19]. As to the function of cannabinoids in the retina, it has been reported that they can modulate voltage-dependent membrane currents in photoreceptor [20], bipolar [21], and ganglionar cells [22]. Moreover, electroretinographic studies in CB1- and CB2-knockout mice indicate that CB2 is the receptor involved in the light response of the retina [23]. Further studies in humans have shown that phytocannabinoids could alter visual acuity, color discrimination, and photosensitivity [24, 25]. The endocannabinoid roles in neuroplasticity and pathophysiological neurodegenerative processes have been widely described [26–28]. Neuroprotective effects of these molecules have been reported in retinal ganglion cells in different models of neuronal insult as oxidative stress [29], optic nerve section [30], and acute ischemia [31], and also in photoreceptor cells in a retinitis pigmentosa model [32]. These findings suggest a substantial role of the ECS in the physiology of the retina and in general vision. However, to date, there is little information about the metabolism of endogenous cannabinoids and its modulation by the photoactivation state of the retina. Thus, the main purpose of the present study was to analyze not only the protein content of cannabinoid receptors and endocannabinoid-related enzymes but also the enzymatic activities involved in 2-AG synthesis (LPAP and DAGL activities) and hydrolysis (MAGL activity) in ROS, to further evaluate if they are modified by light, the physiological stimulus of the retina.

Material and Methods

Materials

[2-^{3}H]Glycerol (2 Ci/mmol) was obtained from Perkin Elmer (Boston, MA, USA). Preblended dry fluor (98% PPO and 2% bis-MSD) was obtained from Research Products International Corp. (Mt. Prospect, IL, USA). Lysophosphatidic acid, 1-oleoyl [oleoyl-9,10-^{3}H(N)]-(54 Ci/mmol) was obtained from American Radiolabeled Chemicals, Inc. (Saint Louis, MO, USA). Oleoyl-L-α lysophosphatidic acid, NEM, and BSA were obtained from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies against CB1 (sc-293419), CB2 (sc-293188), TRPV1 (sc-12498), DAGLα (sc-390409), and MAGL (sc-398942) and against GPR55 (ab203663) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and from Abcam (Cambridge, UK), respectively. Anti-α-tubulin (CP06) and anti-GAPDH (#2118) were from EMD/Biosciences-Calbiochem (San Diego, CA, USA) and

Cell Signaling Technology Inc., (Danvers, MA, USA), respectively. Primary L15 antibody (anti-CB1) was generously supplied by Dr. Ken Mackie. The secondary antibody used for detection of CB1, CB2, DAGL, and MAGL primary antibodies was mouse-IgGk BP-HRP (sc-5116102), for α-tubulin was goat anti-mouse-HRP (sc-2005), and for VR-1 was donkey anti-goat-HRP (sc-2020). They were all obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The secondary antibody used for detection of GPR55 and CB1 (L15) was donkey anti-rabbit-HRP (NA934) from Amersham, GE Healthcare Life Sciences (Pittsburgh, PA, USA). All other chemicals were of the highest purity available.

Rod Outer Segment Isolation

Bovine eyes were obtained from a local slaughterhouse. They were placed on ice within 10 min of the animal's death and subsequently kept in darkness for 2 h. The cornea, lens, and aqueous humor were removed under dim red light. Eyecups were subsequently either kept in darkness or exposed to light (3000 lx for 30 min) at room temperature. In the latter case, eyecups were filled with oxygenated (95% O_2, 5% CO_2) Ames Medium (2 mg/mL glucose, 119.5 mM NaCl, 3.6 mM KCl, 0.1 mM NaH_2PO_4, 0.4 mM Na_2HPO_4, 1.2 mM $MgSO_4$, 1.15 mM $CaCl_2$, 22.6 mM $NaHCO_3$, pH 7.33) [33]. The subsequent procedures for the isolation of ROS from dark-adapted (DROS) or light-exposed (LROS) retinas were performed at 2–4 °C under dim red light. To isolate ROS, retinas were removed and shaken twice in a 40% sucrose solution containing 1 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF, 1 mg/mL aprotinin, 1 mg/mL pepstatin, and 2 mg/mL leupeptin in 70 mM sodium phosphate buffer (pH 7.2) and, in this manner, ROS were detached. The remainder of the retina was sedimented at 2200×*g*for 4 min, and the supernatants containing ROS were diluted 1:2 with sucrose-free buffer and then centrifuged at 35,300×*g* for 30 min. ROS were purified by a discontinuous gradient of sucrose [34] yielding a ROS band retained at the 0.84/1.00 M density interface. The purity of ROS membrane preparations was extensively monitored as was described elsewhere [35]. In addition to our previous study that demonstrates a light-induced movement of transducin and arrestin in or out of ROS [36], in the present work, we analyzed the transducin and arrestin protein level changes in ROS under our experimental conditions (i.e., light and dark conditions). To this end, transducin and arrestin were identified by staining the gel with Commassie Blue, after separating the proteins from DROS and LROS by electrophoresis and by using a prestained protein ladder as a molecular weight marker. We observed a decrease and an increase in transducin and arrestin levels, respectively, in LROS with respect to DROS (data not shown). These light-induced changes are in accordance with results reported by other researchers who evaluated how these proteins move between ROS and RIS under light stimulus [37–39].

In another series of experiments, purified ROS were obtained from dark-adapted retinas (DROS) and subsequently bleached (3000 lx) for 30 min at room temperature (BROS). They were pelleted at 35000×*g* for 30 min at 4 °C to obtain membrane and soluble fractions. MAGL protein levels were determined by western blot in each of these fractions.

Preparation of Radiolabeled Substrates

[2-^{3}H]glycerolphosphatidylcholine (PC) and [2-^{3}H]triacylglycerol (TAG) were synthesized by incubating bovine retinas with [2-^{3}H]glycerol as previously described [40]. 1,2-Diacyl-sn-glycerol (DAG) was obtained from [2-^{3}H]PC after hydrolysis by phospholipase C from *Clostridium welchii* (grade B, Calbiochem, Los Angeles, CA) [41]. Monoacyl-sn-Glycerol (MAG) was obtained from [2-^{3}H]TAG by incubation with pancreatic lipase as specified elsewhere [42]. Lipids were extracted either with n-hexane: 2-propanol (3:2 *v*/*v*) [43] or following Folch et al. (1957) [44]. They were subsequently separated by one-dimensional thin-layer chromatography (TLC) [41, 42]. [^{3}H]DAG and [^{3}H]MAG specific activities, which were determined by gas liquid chromatography (GLC), were 0.05 μCi/μmol and 1.5 μCi/μmol, respectively. The fatty acid composition of each substrate was also determined by GLC and was similar to that previously described [42].

DAGL Activity Assay

This enzymatic assay was performed using [^{3}H]DAG as a substrate in 50 mM MOPS buffer (pH 7.4) containing 0.25% fatty acid–free bovine serum albumin (BSA) in a final volume of 100 μL [43]. The substrate [^{3}H]DAG was prepared by sonicating it with equimolecular concentrations of LPC in 50 mM MOPS buffer (pH 7.4). The assay was initiated by adding [^{3}H]DAG (2 mM, 20,000 DPM) to DROS and LROS preparations (150 μg of protein) and incubated for 1 h.

LPAP Activity Assay

LPAP activity was achieved by incubating ROS with unlabeled 1-oleoyl LPA and 1-oleoyl [oleoyl-9,10-3H(N)]-LPA (20 μM, 6000 DPM) in 100 mM Tris-HCl (pH 7.4), 1.2 mM DTT, 2 mM EDTA, in a final volume of 100 μL [45]. After 10 min of pre-incubation of ROS (100 μg of protein) with 4.4 mM NEM, substrate was added and incubated for 15 min. Substrate was prepared in 100 mM Tris-HCl, pH 7.4, containing 0.1% BSA [46].

MAGL Activity Assay

MAGL activity was assessed by incubating ROS in a buffer solution of Tris-HCl (50 mM, pH 7.5) containing 1 mM EDTA in a final volume of 100 μL. [^{3}H]-MAG (10 μM, 2500 DPM) was prepared by sonicating it with free fatty acid BSA (1.25 mg/mL) [46, 47] and incubated with ROS for 15 min.

All enzymatic reactions were conducted at 37 °C under dim red light. The reactions were stopped by adding either chloroform:methanol (2:1, *v*/*v*) or chloroform:methanol (1:1, *v*/*v*). Lipid products were subsequently extracted with chloroform:methanol (2:1, *v*/*v*) [44].

Blank preparations were identical to those of each enzymatic assay except that ROS proteins were denaturalized by the addition of chloroform:methanol (2:1, *v*/*v*) before use.

Separation and Quantification of Enzymatic Reaction Products

MAG (DAGL product) was separated by TLC on a silica gel G plate and developed with hexane:diethyl ether:acetic acid (45:55:1.5, *v*/*v*) [48]. LPAP products, MAG, and free fatty acids were chromatographed by TLC on a silica gel H plate and developed with chloroform:acetone:methanol:acetic acid:water (30:40:10:10:4, *v*/*v*) up to the middle of the plate. The chromatogram was subsequently re-chromatographed up to the top of the plate using hexane:diethyl ether:acetic acid (45:55:1.5, *v*/*v*) as developing solvent. Chromatograms were visualized by exposure to iodine vapors and scraped off for counting by liquid scintillation. Glycerol (MAGL product) was obtained in the upper phase after interrupting the enzymatic reaction. The aqueous phase containing radiolabel glycerol was concentrated to dryness and counted by liquid scintillation. Radiolabel samples were counted after the addition of 0.25 mL water and 5 mL of 0.5% preblended dry fluor (98% PPO and 2% bis-MSD) in toluene/Arkopal N-100 (4:1, *v*/*v*).

SDS-PAGE and Immunoblot

Proteins from purified DROS or LROS and from DROS and BROS membrane and soluble fractions were boiled in Laemmli buffer. They were subsequently resolved in SDS-PAGE using 10% acrylamide/bis-acrylamide gels in Tris-glycine buffer according to Laemmli (1970) [49]. Resolved proteins were transferred to Immobilon-P PVDF membranes using a Mini Trans-Blot cell electro blotter (BIO-RAD, Hercules, CA, USA) for 75 min. Membranes were blocked for 1 h with 5% non-fat milk in Tris-buffered saline (20 mM Tris-HCl, 150 mM NaCl) pH 7.5, containing 0.05% Tween 20 (TTBS). Incubations with primary antibodies anti-DAGL (1:200), anti-MAGL (1:500), anti-CB1 (1:500 or 1:3000), anti-CB2 (1:500), anti-GPR55 (1:500), anti-VR1 (1:500), anti-α-tubulin (1:3000), and anti-GAPDH (1:1000) were carried out at 4 °C overnight. Membranes were washed with TTBS and subsequently exposed to the appropriate HRP-conjugated secondary antibody (anti-rabbit, anti-goat, or anti-mouse) for 3 h. The membranes were rewashed with TTBS, and immunoreactive bands were detected by enhanced chemiluminescence using standard X-ray film (Kodak X-Omat AR). Precision Plus Protein Standards Kaleidoscope (#161-0375, BIO-RAD, Hercules, CA, USA) was used as a molecular weight marker.

Protein content was determined using DC Protein Assay (#500-0116, BIO-RAD, Hercules, CA, USA) according to Lowry et al. (1951) [50].

Statistical Analysis

Three pools (22 retinas per pool) were prepared, and each one was used to assay one and three replicates per condition for western blot and enzymatic activity determinations, respectively. Each pool was considered as a block in a randomized block design. To determine differences between DROS and LROS, randomized block analysis of variance (ANOVA) with Fisher's LSD post-test was performed. Statistical significance was set at $p < 0.05$, thus considering 0.05 global error (α). All figures, except Fig. 4b, are given as mean values ± standard error of the mean (S.E.M).

Results

DAGL and LPAP Activities in Rod Outer Segments from Retinas Under Dark (DROS) and Light (LROS) Conditions

The DAGL and LPAP enzymes involved in the formation of 2-AG were assayed in DROS and LROS. Under the optimal assay conditions for each enzyme, the contribution of DAGL and LPAP to MAG production in DROS was similar (Fig. 1a and b). While DAGL activity was increased by 103% (Fig. 1a) in LROS with respect to DROS, LPAP activity was not modified by the illumination state of the retina (Fig. 1b).

MAGL Activity in Rod Outer Segments from Retinas Under Dark (DROS) and Light (LROS) Conditions

MAGL activity, the principal enzyme that hydrolyzes MAG, was analyzed using exogenous MAG as substrate. This activity shows an important increase (69%) in LROS with respect

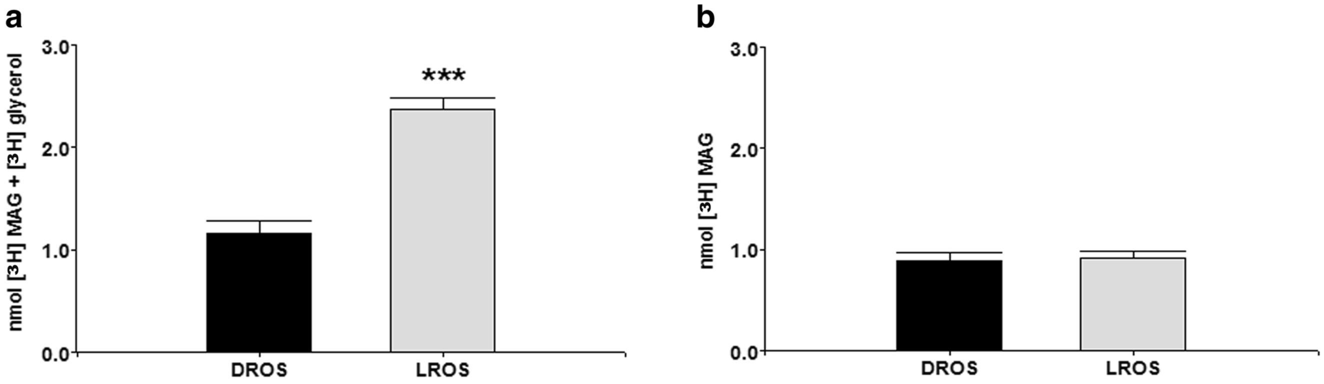

Fig. 1 DAGL (**a**) and LPAP (**b**) activities in rod outer segments from retinas under dark (DROS) and light (LROS) conditions. Enzymatic activities were determined using, as an enzyme source, either purified ROS from dark-adapted retinas (DROS) or bleached retinas (LROS) whose optic cups were exposed to light (3000 lx for 30 min) at room temperature as specified in section 2.2. ROS preparation and the enzymatic assays were carried out under dim red light. DAGL and LPAP activities were analyzed using [2-^{3}H]DAG and 1-oleoyl [oleoyl-9,10-^{3}H(N)]-LPA as substrates, respectively. These enzymatic assays are specified in sections 2.4 and 2.5 of the Material and Methods section. Incubation products were subsequently extracted and separated by thin-layer chromatography and visualized after exposure to iodine vapor. The bands corresponding to enzymatic products were scraped and quantitated by liquid scintillation spectroscopy. Results represent the mean ± S.E.M ($n = 9$, ***$p < 0.001$ with respect to DROS)

to DROS (Fig. 2a). We observed that MAG formed by DAGL activity was additionally metabolized to glycerol. Although an increase (94%) was apparently observed under light condition (Fig. 2b), MAG (63%) and glycerol (37%) percentages with respect to the total DAG degradation were similar under both conditions (Fig. 2b, insert).

Light Effect on PLCβ_1, DAGLα, and MAGL Protein Levels in Rod Outer Segments

The synthetic and hydrolytic enzymes and their isoforms involved in 2-AG metabolism were analyzed by western blot employing the respective antibodies against PLCβ_1 and DAGLα (Fig. 3) and MAGL (Fig. 4a and b). PLCβ_1 (Fig. 3a) and DAGLα (Fig. 3b) protein levels were increased (99%) and decreased (15%), respectively, in LROS with respect to DROS. MAGL protein level was diminished by 30% in ROS from retinas under light stimulus (Fig. 4a). As MAGL is a cytosolic and/or a membrane-associated enzyme [12, 47], we also analyzed if MAGL distribution between these fractions was modified by light. To this end, MAGL levels were determined in DROS and BROS (ROS obtained in darkness and subsequently illuminated) membrane and soluble fractions. Our results show a tendency of MAGL to increase in soluble BROS (Fig. 4b).

Light Effect on CB1, CB2, GPR55, and TRPV1 Protein Levels in Rod Outer Segments

Protein levels of cannabinoid-binding receptors were also analyzed by western blot using the corresponding antibody

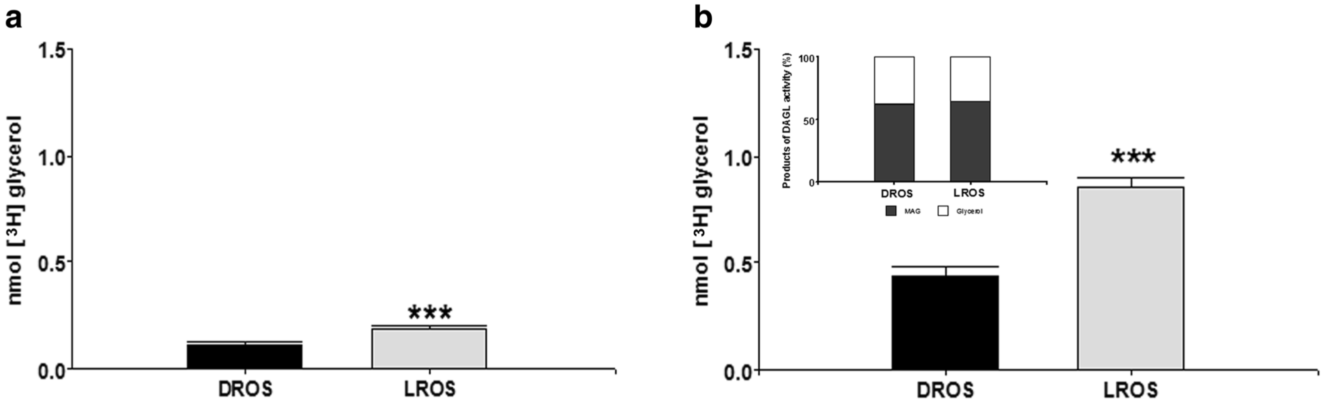

Fig. 2 MAGL activity in rod outer segments from retinas under dark (DROS) and light (LROS) conditions. MAGL activity was assayed using as substrate [^{3}H]MAG added exogenously (**a**) or [^{3}H]MAG endogenously (**b**) generated from the enzymatic hydrolysis of DAGL on [^{3}H]DAG. All assays were carried out in the presence of DROS or LROS as is specified in section 2.6 of the Material and Methods section. MAGL product, [^{3}H]glycerol, which was obtained in the upper phase, was concentrated to dryness and counted by liquid scintillation. The insert of Fig. 2b represents the percentage of glycerol and MAG generated by MAGL coupled to DAGL activity, in DROS and LROS. Results represent the mean ± S.E.M ($n = 9$, ***$p < 0.001$ with respect to DROS)

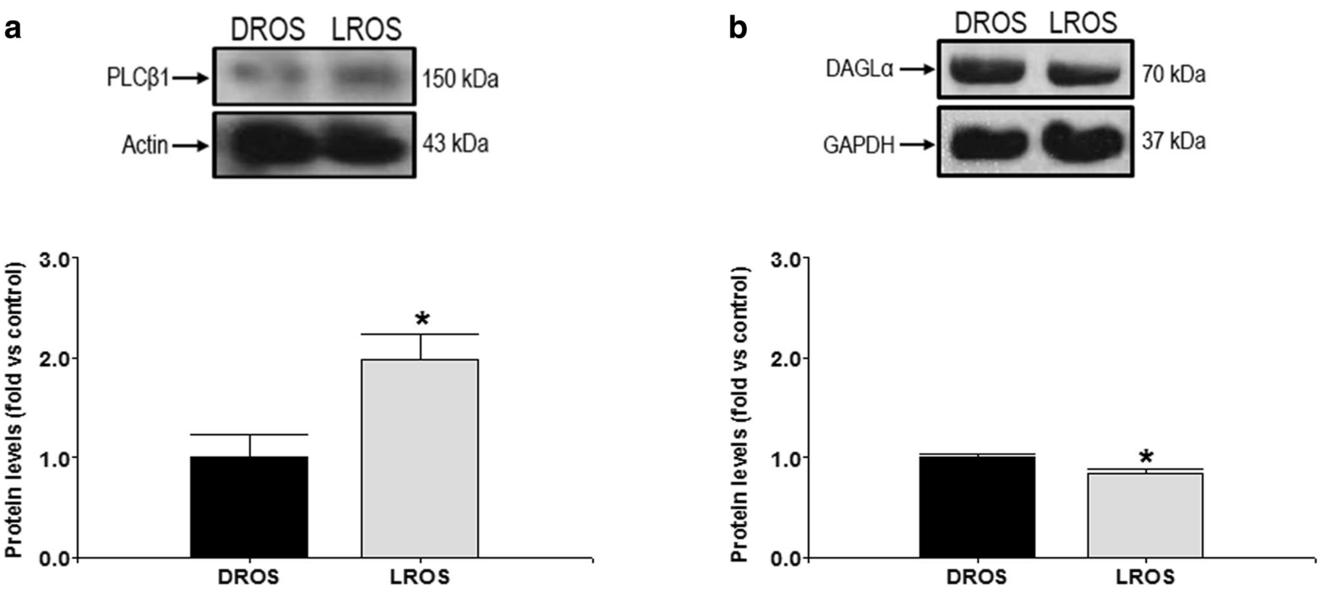

Fig. 3 Light effect on PLCβ$_1$ (**a**) and DAGLα (**b**) protein levels in rod outer segments. Immunoblot analyses were performed in DROS and LROS. Proteins (100 μg) were boiled in Laemmli buffer, resolved in a 10% SDS-PAGE, and transferred to a PDVF membrane for further WB assays. Membranes were blocked and incubated with primary and secondary antibodies as detailed the Material and Methods section. Immunoreactive bands were detected by enhanced chemiluminescence. Numbers on the right indicate molecular weights. The bar graph shows relative density corresponding to protein level expressed as a ratio of loading control (actin or GAPDH). Results represent the mean ± S.E.M ($n = 3$, *$p < 0.05$ with respect to DROS)

against CB1, CB2, GPR55, and TRPV1 (Fig. 4). CB1 and CB2 protein levels were increased by 87% (Fig. 5a) and 69% (Fig. 5b), respectively, in LROS with respect to DROS. On the other hand, whereas GPR55 protein level was diminished (30%) (Fig. 5c) under light condition, that from TRPV1 remained unmodified (Fig. 5d).

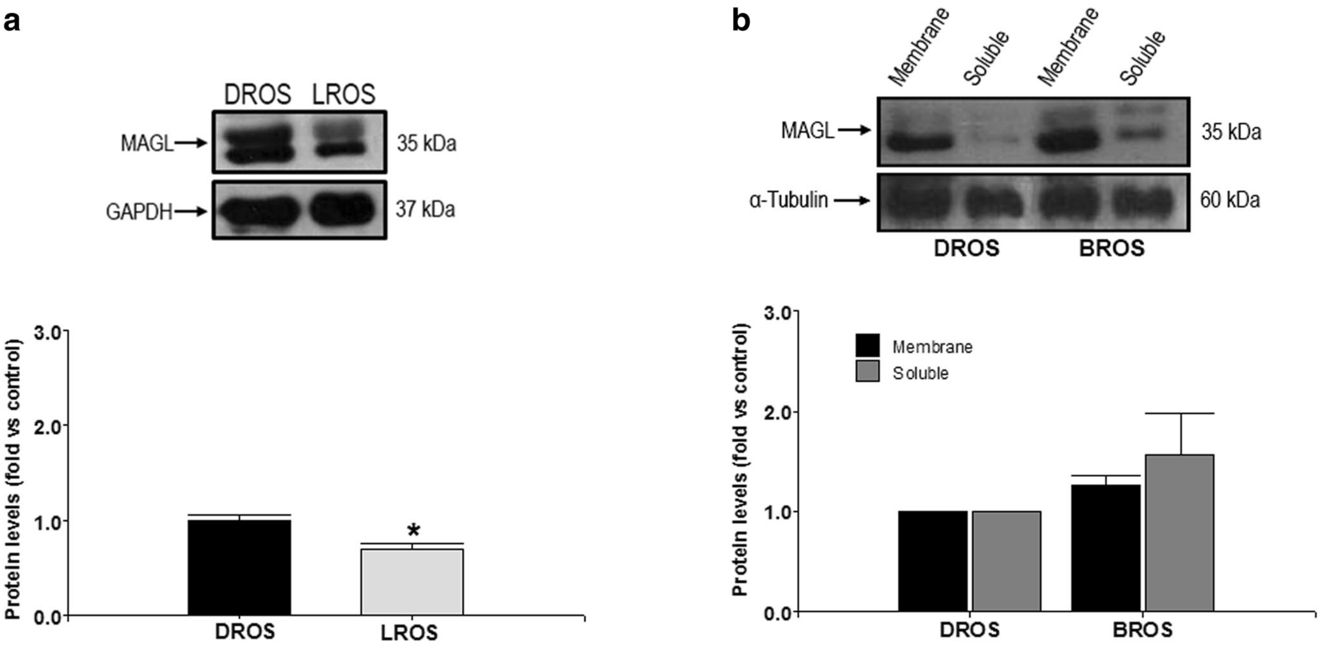

Fig. 4 Light effect on MAGL protein levels in rod outer segments. Immunoblot analyses were performed in DROS and LROS (**a**) and in the membrane and soluble fractions from DROS and BROS (**b**). Proteins (100 μg) (**a**) and 80 μg (membrane fraction) and 15 μg (soluble fraction) (**b**) were boiled in Laemmli buffer, resolved in a 10% SDS-PAGE and transferred to a PDVF membrane for further WB assays. Membranes were blocked and incubated with primary and secondary antibodies as detailed in the Material and Methods section. Immunoreactive bands were detected by enhanced chemiluminescence. Numbers on the right indicate molecular weights. The bar graph shows relative density corresponding to protein content expressed as a ratio of loading control (GAPDH or tubulin). Results represent (**a**) the mean ± S.E.M ($n = 3$, *$p < 0.05$ with respect to DROS; (**b**) the mean ± S.D. ($n = 3$)

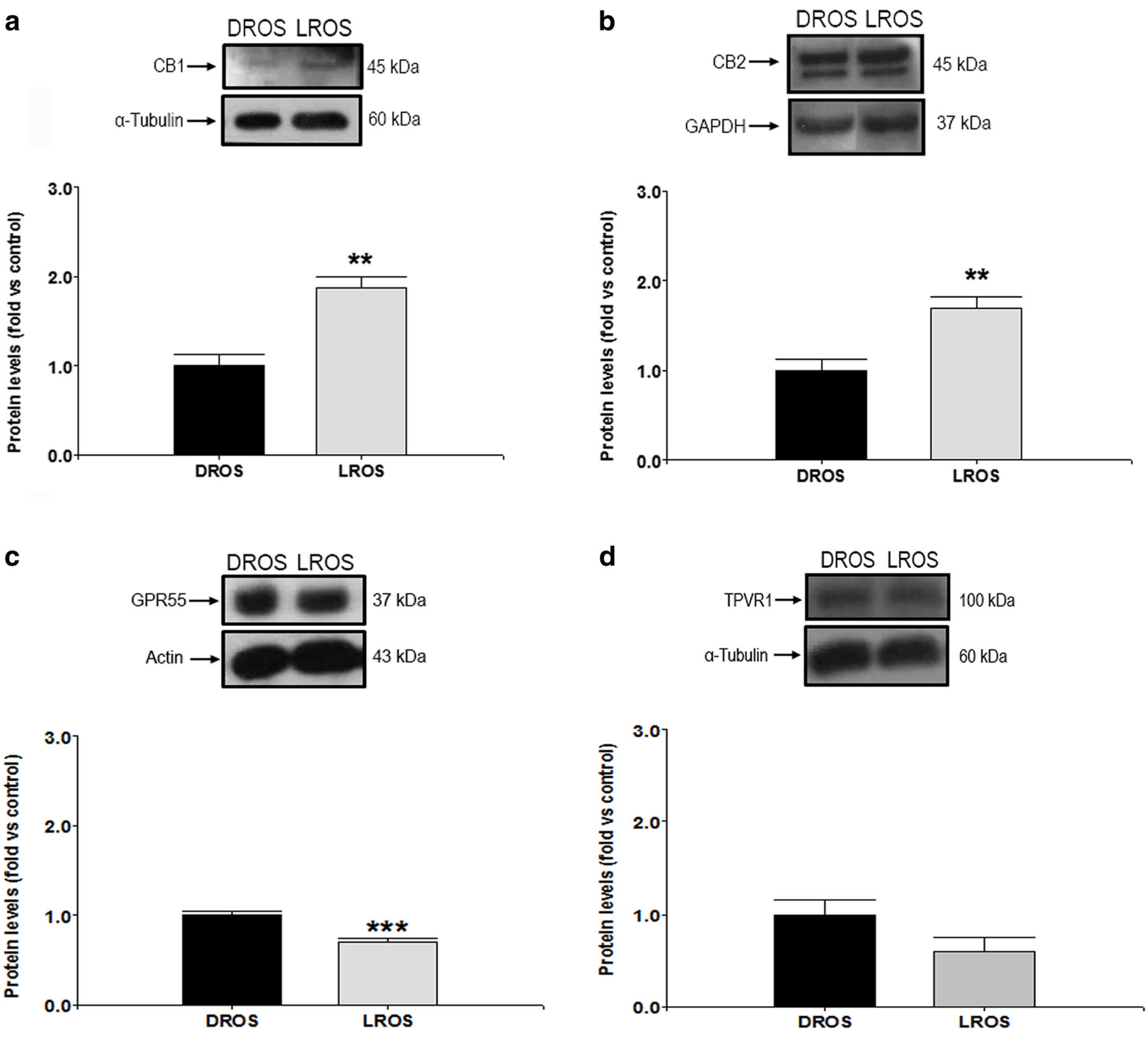

Fig. 5 Light effect on CB1 (**a**), CB2 (**b**), GPR55 (**c**), and TRPV1 (**d**) protein levels in rod outer segments. Immunoblot analyses were performed as specified in Fig. 3. Numbers on the right indicate molecular weights. The bar graph shows relative density corresponding to protein level expressed as a ratio of loading control (actin, GAPDH, or α-tubulin). Results represent the mean ± S.E.M ($n = 3$, $^{***}p < 0.001$, $^{**}p < 0.01$ with respect to DROS)

Discussion

The retina is an extension of the CNS whose primary function is to process light and color captured by photoreceptor neurons. This information is transmitted to the visual cortex and is processed in order to generate a visual image. The outer segment compartment of the photoreceptor cell is responsible for the initial events of vision at low light levels and it contains large amounts of proteins that are involved in light detection mechanisms as well as in the generation of visual signaling [2, 51]. The ECS localization (endocannabinoids, receptors, and enzymes) has been studied in the retina of various species (from salamander to primates, and humans) by means of different techniques, mainly immunohistochemistry [20, 52, 53]. Although these studies strongly suggest that the ECS actively participates in visual processing and that light modulates a number of enzymes that generate second lipid messengers in ROS (reviewed in Giusto et al. 2010) [3], no research has yet focused on endocannabinoid metabolism. It has been reported that endocannabinoids attenuate brain damages induced by a variety of insults. In this respect, it has been proposed that the ECS is upregulated in order to diminish CNS damages in trauma and in neurodegenerative diseases, in which endocannabinoids behave as neuroprotectors [54–56]. Taking into account that the retina is considered part of the CNS and that the ECS is involved in many neuroprotection processes, the present paper opens new avenues to assess ECS distribution in ROS and its role as a neuroprotector in physiopathological processes, such as retinal damage induced by high light intensity.

The synthesis of 2-AG is produced basically by the concerted action of PLC/DAGL or by LPAP activities on PIP_2 and LPA, respectively. Our results show that both enzymatic activities could contribute to 2-AG production in ROS. DAGL is known to be present in bovine neural tissue [43] and two isoforms (α and β) of this enzyme have been cloned and characterized in the brain [57]. It has been shown that DAGL hydrolytic activity may vary upon the acyl chain length and degree of saturation of the fatty acids of the DAG used as substrate [43, 57]. The composition of the $[^3H]$-DAG used in our assays contained 34%, 19%, 18%, and 17% of 16:0, 18:0, 18:1, and 22:6, respectively, with only 5% of 20:4 [40, 42]. Nevertheless, previous research has demonstrated that bovine brain DAGL hydrolyzes DAG with a composition similar to our substrate and its specific activity was equal to that when 18:0/20:4-DAG was used [43].

Interestingly, the results of the present paper show that only DAGL contribution to 2-AG synthesis was stimulated by light. Previous studies from our laboratory demonstrated that in ROS from the bovine retina, DAG produced from phosphatidic acid by lipid phosphate phosphatases (LPP) was additionally metabolized to monoacylglycerol, thus suggesting the presence of DAGL activity coupled to LPP [40, 58]. We also observed that the bleaching of the retina diminished the production of MAG from PA [59, 60], which appears to be indicative of an inhibitory effect on the DAGL coupled to LPP activity. This differential light effect on DAGL could be probably due to differences in the substrate availability as DAG formation depended on phosphatidic acid hydrolysis by LPP. Light-induced diminution in LPP activity produces a lower DAG concentration for DAGL activity and it consequently affects MAG production [59]. In the present paper, however, we used an exogenous DAG with similar fatty acid composition and ensured that its concentration was the same for both conditions. It should be noted that in the present study the light effects observed in ROS on protein levels and activities of the ECS enzymes and receptors were due only to the bleaching of the retina since both ROS preparations and enzymatic assays were performed under dim red light for dark and light conditions.

2-AG hydrolysis was analyzed by determining MAGL activity, the main enzyme that degrades it [12]. MAGL activity in ROS was observed not only using the exogenous substrate but also in MAG produced when DAGL activity was assayed. The production of glycerol in the latter case was found to exceed three times more than that from the exogenous substrate, thus suggesting that DAGL and MAGL behave like an enzymatic complex where the product of the first enzyme is more accessible to the active site of the second enzyme and/or that the binding of the enzyme to the lipid bilayer is a crucial issue in MAGL activity as was demonstrated for other lipid metabolism enzymes [61]. Interestingly, the effect of light stimulus was only observed when the activity was determined using the exogenous substrate. Glycerol formation by DAGL/MAGL showed an apparent increase in LROS as a consequence of the stimulus in DAGL activity, which generates a higher availability of the substrate (MAG) for MAGL action. According to this, the same percentage of MAG (65%) and glycerol (35%) was observed in both DROS and LROS. Moreover, the highest availability of MAG with no changes in MAGL activity could mean that the substrate concentration was already saturating the enzyme. This could be possible because MAG concentration generated from DAG hydrolysis was equivalent to 12 μM in DROS (similar to that used with the exogenous substrate in DROS and LROS), whereas in LROS it was 24 μM. It can thus be concluded that although both 2-AG synthesis and hydrolysis are stimulated in LROS, the effect on 2-AG synthesis is significantly higher, thus indicating a greater availability of this endocannabinoid in ROS when the retina is bleached.

On the other hand, the content of PLC β1, an enzyme that precedes the action of DAGL, was observed to be strongly increased, whereas DAGL and MAGL protein levels were diminished in the bleached retina. The enzymes involved in the synthesis of 2-AG (DAGL and LPAP) and the receptors to which cannabinoids bind are integral membrane proteins, both of which can only be extracted by membrane disruption [6, 42, 57, 62, 63]. However, MAGL, the hydrolytic 2-AG enzyme, is considered both a cytosolic and a membrane-associated enzyme [12, 47]. The crystal structure of human MAGL shows it as an amphitropic enzyme due to the presence of a hydrophobic helix. This helix allows soluble MAGL either to get in close contact with or to anchor in the membrane in order to reach its lipophilic substrates [64]. Our results on MAGL levels in the membrane and soluble fraction in BROS with respect to those in DROS show a tendency of MAGL to increase in soluble BROS. This could be indicative of a light-induced MAGL redistribution pattern from the membrane to the soluble fraction although no loss of membrane MAGL protein was observed. This absence of membrane MAGL–associated loss could be due to the fact that protein content in the membrane fraction is higher than that in the soluble fraction and therefore minimum variations in membrane protein levels pass unnoticed. We observed that the bleaching of the retina generates a slight decrease in MAGL level probably by its migration to the RIS. In parallel, our results regarding BROS suggest a MAGL detachment from the membrane to the soluble fraction. Any of these mechanisms could prevent a decrease in 2-AG availability [12, 47].

Our results regarding the protein content and the activity of the enzymes involved in 2-AG metabolism in addition to the stimulus exerted by light in PLC enzymatic activity [65] indicate that under light conditions a higher availability of 2-AG

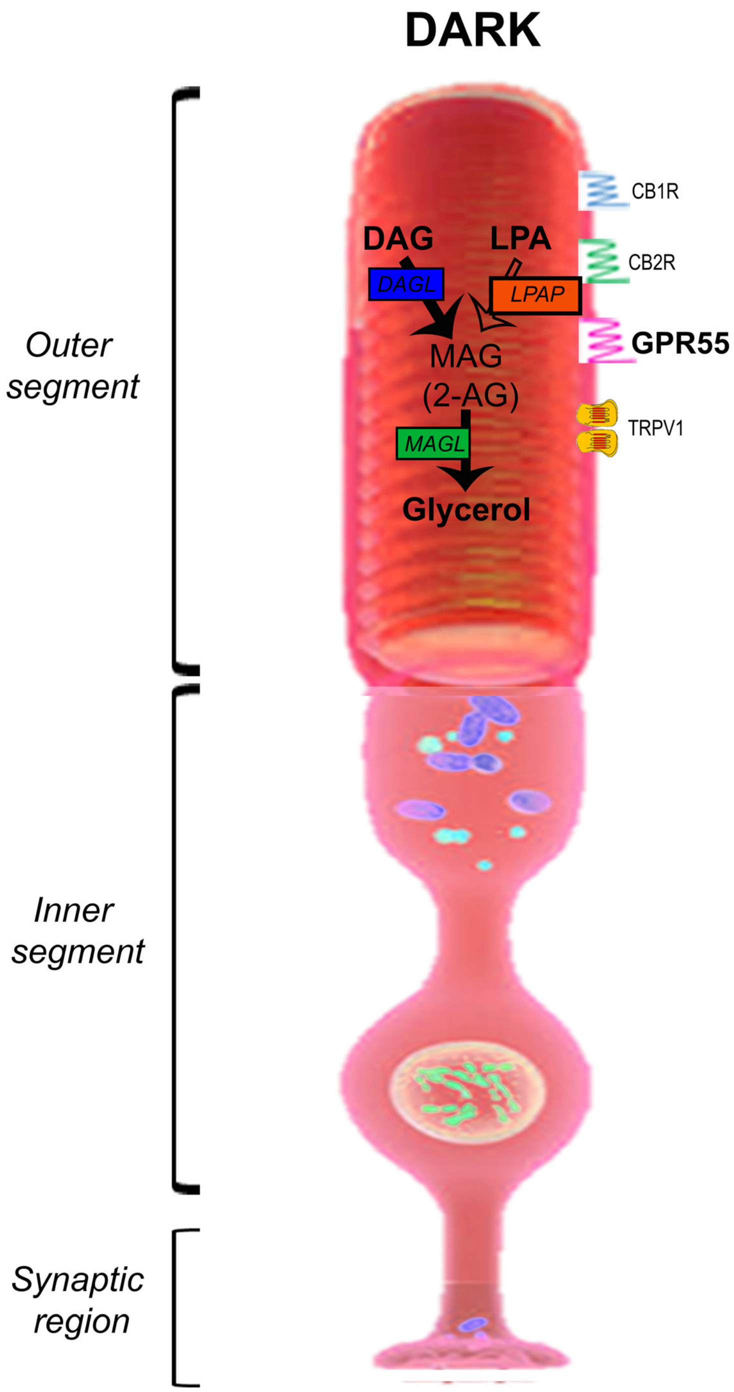

Fig. 6 Light-related changes in the endocannabinoid system in rod outer segments. The relative size of arrows indicates the predominance of MAG (2-AG) in its synthesis and/or breakdown. The relative size of the receptor names indicates its predominance. LPA 1-oleoyl lysophosphatidic acid, DAG diacylglycerol, MAG monoacylglycerol, 2-AG 2-arachydonoyl glycerol. CB1 and CB2 cannabinoid receptors 1 and 2, GPR55 G protein–coupled receptor 55, TRPV1 transient receptor potential cation channel. The enzyme name is in italic script. LPAP lysophosphatidic acid phosphohydrolase, DAGL diacylglycerol lipase, MAGL monoacylglycerol lipase

appears to be necessary. These effects could be mediated either by transducin activation or by light-mediated photoassociation of some factor that stimulates the enzymatic activities in order to produce this endocannabinoid. It has been reported that light causes an increase in the amplification of the rod photoresponse, allowing massive light-dependent translocation of proteins involved in signal transduction, such as arrestin, transducin, and recoverin between outer and inner segments of rods. This mechanism, which desensitizes rods under conditions of intensive illumination, contributes to rod light adaptation [38, 66, 67]. In this context, any soluble or peripheral protein detached from ROS and/or protein redistribution produced by bleaching could modify endocannabinoid metabolism. Additional experiments will be necessary to elucidate this. Our results also reveal not only the presence of CB1 and CB2 receptors in ROS but also their increased protein level under the illumination state of the retina. The movement of proteins from their place of synthesis in the inner segment to the place where phototransduction occurs is an actively explored field of research. It is an issue which has acquired relevance due to the fact that an altered protein trafficking leads to the death of the photoreceptor. Rhodopsin has a motif in its terminal carboxyl that allows its recognition by means of transport proteins which move towards the connecting cilium for its arrival at ROS [68, 69]. Approximately 2000 opsin molecules are transported per minute from RIS to ROS [70]. Recognition sites have also been described for other ROS integral proteins [71, 72]. CB1 and CB2 receptors are also membrane integral proteins, consequently, their transport could be similar to that which occurs for opsin and other transmembrane proteins of ROS. The increase in the level of CB1 and CB2 receptors by light could be indicative of a greater transport of these receptors towards ROS to respond to higher availability of 2-AG under this condition. In support of this, it has been reported that the redistribution of arrestin and transducin between RIS and ROS by light is accompanied by a large flow of proteins between these compartments [73].

The apparent upregulation of the endocannabinoid system in ROS could contribute to the phototransduction process as a modulator and/or protect the photoreceptor against a possible insult due to a high-intensity light exposure. Our results also demonstrate a higher increase of CB1 than CB2 protein content under light stimulus. In this respect, electroretinographic studies in mice lacking CB1 or CB2 receptors indicate that both receptors contribute to visual functions [23] as well as to primate retinal function [74]. Based on the fact that CB1 antagonist SR141716A attenuates photoreceptor loss, recent research has concluded that the CB1 receptor has an important role in retinitis pigmentosa as well as in light-induced degeneration [75, 76].

It has been reported that orphan G protein–coupled receptor 55 (GPR55) is mostly expressed in rods of monkey retina, suggesting a function of this receptor in scotopic vision [18]. Although lysophosphatidylinositol has been postulated as the endogenous ligand of GPR55 [77], the latter has also been suggested to be a cannabinoid receptor based on its activation by cannabinoid ligands [78]. Another cannabinoid-like receptor present in the retina is the transient receptor potential vanilloid 1 (TRPV1), which binds endocannabinoids, such as AEA and N-arachidonoyl dopamine [79]. Our results demonstrate that light diminishes GPR55 while TRPV1 protein level remains unmodified. The different receptor protein content in ROS under light condition seems to indicate that each of these receptors has a unique function in the retina and in visual processing. Recently, Bouskila et al. (2016) have reported that GPR55 activation by a specific agonist modulates the scotopic b-wave of electroretinogram in vervet monkey, evidencing the role of this receptor in scotopic vision. In view of this, the decrease in the protein level of GPR55 observed in our study under light conditions could be related to the role of this receptor that seems to be functional in dark conditions [18, 80].

Our findings summarized in Fig. 6 support the participation of the endocannabinoid system in response to light. This paper describes for the first time how light modulates not only the protein level of cannabinoid receptors but also the enzymes and the enzymatic activities involved in endocannabinoid metabolism in ROS. Further experiments are nonetheless still needed in order to clarify the precise role of ECS in scotopic vision.

Author's Contributions SJP and VLG conceived and designed the experiments and analyzed the data. ECHA and VLG performed the experiments. SJP wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

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Compliance with Ethical Standards

Consent for Publication All authors have read the manuscript and agreed to its content. The article is original, has not already been published in a journal, and is not currently under consideration by another journal.

Competing Interests The authors declare that they have no conflicts of interest.

References

1. Laties AM, Bok D, Liebman P (1976) Procion yellow: a marker dye for outer segment disc patency and for rod renewal. Exp Eye Res 23:139–148
2. Arshavsky VY, Burns ME (2012) Photoreceptor signaling: supporting vision across a wide range of light intensities. J Biol Chem 287:1620–1626
3. Giusto NM, Pasquare SJ, Salvador GA, Ilincheta de Boschero MG (2010) Lipid second messengers and related enzymes in vertebrate rod outer segments. J Lipid Res 51:685–700
4. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A et al (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949
5. Sugiura T, Kishimoto S, Oka S, Gokoh M (2006) Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog Lipid Res 45:405–446
6. Howlett AC (2002) The cannabinoid receptors. Prostaglandins Other Lipid Mediat 68-69:619–631
7. Okamoto Y, Wang J, Morishita J, Ueda N (2007) Biosynthetic pathways of the endocannabinoid anandamide. Chem Biodivers 4:1842–1857
8. Maurelli S, Bisogno T, De PL, Di LA, Marino G, Di M, V (1995) Two novel classes of neuroactive fatty acid amides are substrates

for mouse neuroblastoma ‘anandamide amidohydrolase’. FEBS Lett 377:82–86

9. Prescott SM, Majerus PW (1983) Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate. J Biol Chem 258:764–769
10. Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388:773–778
11. Nakane S, Oka S, Arai S, Waku K, Ishima Y, Tokumura A, Sugiura T (2002) 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. Arch Biochem Biophys 402:51–58
12. Dinh TP, Kathuria S, Piomelli D (2004) RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol Pharmacol 66:1260–1264
13. Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 20:441–458
14. Savinainen JR, Saario SM, Laitinen JT (2012) The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol (Oxford) 204:267–276
15. Hu SS, Arnold A, Hutchens JM, Radicke J, Cravatt BF, Wager-Miller J, Mackie K, Straiker A (2010) Architecture of cannabinoid signaling in mouse retina. J Comp Neurol 518:3848–3866
16. Yazulla S (2008) Endocannabinoids in the retina: from marijuana to neuroprotection. Prog Retin Eye Res 27:501–526
17. Lopez EM, Tagliaferro P, Onaivi ES, Lopez-Costa JJ (2011) Distribution of CB2 cannabinoid receptor in adult rat retina. Synapse 65:388–392
18. Bouskila J, Javadi P, Casanova C, Ptito M, Bouchard JF (2013) Rod photoreceptors express GPR55 in the adult vervet monkey retina. PLoS One 8:e81080
19. Henstridge CM (2012) Off-target cannabinoid effects mediated by GPR55. Pharmacology 89:179–187
20. Straiker A, Sullivan JM (2003) Cannabinoid receptor activation differentially modulates ion channels in photoreceptors of the tiger salamander. J Neurophysiol 89:2647–2654
21. Fan SF, Yazulla S (2005) Reciprocal inhibition of voltage-gated potassium currents (I K(V)) by activation of cannabinoid CB1 and dopamine D1 receptors in ON bipolar cells of goldfish retina. Vis Neurosci 22:55–63
22. Lalonde MR, Chauhan BC, Tremblay F (2006) Retinal ganglion cell activity from the multifocal electroretinogram in pig: optic nerve section, anaesthesia and intravitreal tetrodotoxin. J Physiol 570:325–338
23. Cecyre B, Zabouri N, Huppe-Gourgues F, Bouchard JF, Casanova C (2013) Roles of cannabinoid receptors type 1 and 2 on the retinal function of adult mice. Invest Ophthalmol Vis Sci 54:8079–8090
24. Dawson WW, Jimenez-Antillon CF, Perez JM, Zeskind JA (1977) Marijuana and vision–after ten years’ use in Costa Rica. Invest Ophthalmol Vis Sci 16:689–699
25. Kiplinger GF, Manno JE, Rodda BE, Forney RB (1971) Dose-response analysis of the effects of tetrahydrocannabinol in man. Clin Pharmacol Ther 12:650–657
26. Pascual AC, Martin-Moreno AM, Giusto NM, de Ceballos ML, Pasquare SJ (2014) Normal aging in rats and pathological aging in human Alzheimer’s disease decrease FAAH activity: modulation by cannabinoid agonists. Exp Gerontol 60:92–99
27. Pascual AC, Gaveglio VL, Giusto NM, Pasquare SJ (2014) Cannabinoid receptor-dependent metabolism of 2-arachidonoylglycerol during aging. Exp Gerontol 55:134–142
28. Scotter EL, Abood ME, Glass M (2010) The endocannabinoid system as a target for the treatment of neurodegenerative disease. Br J Pharmacol 160:480–498
29. El-Remessy AB, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai NJ, Roon P, Caldwell RB, Caldwell RW et al (2003) Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol 163:1997–2008
30. Slusar JE, Cairns EA, Szczesniak AM, Bradshaw HB, Di PA, Kelly ME (2013) The fatty acid amide hydrolase inhibitor, URB597, promotes retinal ganglion cell neuroprotection in a rat model of optic nerve axotomy. Neuropharmacology 72:116–125
31. Pinar-Sueiro S, Zorrilla Hurtado JA, Veiga-Crespo P, Sharma SC, Vecino E (2013) Neuroprotective effects of topical CB1 agonist WIN 55212-2 on retinal ganglion cells after acute rise in intraocular pressure induced ischemia in rat. Exp Eye Res 110:55–58
32. Lax P, Esquiva G, Altavilla C, Cuenca N (2014) Neuroprotective effects of the cannabinoid agonist HU210 on retinal degeneration. Exp Eye Res 120:175–185
33. AMES A, III HASTINGSAB (1956) Studies on water and electrolytes in nervous tissue. I. Rabbit retina: methods and interpretation of data. J Neurophysiol 19:201–212
34. Kuhn H (1982) Light-regulated binding of proteins to photoreceptor membranes and its use for the purification of several rod cell proteins. Methods Enzymol 81:556–564
35. Roque ME, Giusto NM (1995) Phosphatidylethanolamine N-methyltransferase activity in isolated rod outer segments from bovine retina. Exp Eye Res 60:631–643
36. Salvador GA, Giusto NM (2006) Phospholipase D from photoreceptor rod outer segments is a downstream effector of RhoA: evidence of a light-dependent mechanism. Exp Eye Res 83:202–211
37. Elias RV, Sezate SS, Cao W, McGinnis JF (2004) Temporal kinetics of the light/dark translocation and compartmentation of arrestin and alpha-transducin in mouse photoreceptor cells. Mol Vis 10:672–681
38. Sokolov M, Lyubarsky AL, Strissel KJ, Savchenko AB, Govardovskii VI, Pugh EN Jr, Arshavsky VY (2002) Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. Neuron 34:95–106
39. Sokolov M, Strissel KJ, Leskov IB, Michaud NA, Govardovskii VI, Arshavsky VY (2004) Phosducin facilitates light-driven transducin translocation in rod photoreceptors. Evidence from the phosducin knockout mouse. J Biol Chem 279:19149–19156
40. Pasquare de Garcia SJ, Giusto NM (1986) Phosphatidate phosphatase activity in isolated rod outer segment from bovine retina. Biochim Biophys Acta 875:195–202
41. Gaveglio VL, Pasquare SJ, Giusto NM (2011) Metabolic pathways for the degradation of phosphatidic acid in isolated nuclei from cerebellar cells. Arch Biochem Biophys 507:271–280
42. Pascual AC, Gaveglio VL, Giusto NM, Pasquare SJ (2013) Aging modifies the enzymatic activities involved in 2-arachidonoylglycerol metabolism. Biofactors 39:209–220
43. Rosenberger TA, Farooqui AA, Horrocks LA (2007) Bovine brain diacylglycerol lipase: substrate specificity and activation by cyclic AMP-dependent protein kinase. Lipids 42:187–195
44. Folch J, Lees M, Slone Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
45. Fleming IN, Yeaman SJ (1995) Subcellular distribution of N-ethylmaleimide-sensitive and -insensitive phosphatidic acid phosphohydrolase in rat brain. Biochim Biophys Acta 1254:161–168
46. Baker RR, Chang H (2000) A metabolic path for the degradation of lysophosphatidic acid, an inhibitor of lysophosphatidylcholine

lysophospholipase, in neuronal nuclei of cerebral cortex. Biochim Biophys Acta 1483:58–68

47. Blankman JL, Simon GM, Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14:1347–1356
48. Giusto NM, Bazan NG (1979) Phospholipids and acylglycerols biosynthesis and 14CO2 production from [14C]glycerol in the bovine retina: the effects of incubation time, oxygen and glucose. Exp Eye Res 29:155–168
49. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
50. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
51. Fain GL, Matthews HR, Cornwall MC, Koutalos Y (2001) Adaptation in vertebrate photoreceptors. Physiol Rev 81:117–151
52. Bouskila J, Javadi P, Elkrief L, Casanova C, Bouchard JF, Ptito M (2016) A comparative analysis of the endocannabinoid system in the retina of mice, tree shrews, and monkeys. Neural Plast 2016: 3127658
53. Straiker AJ, Maguire G, Mackie K, Lindsey J (1999) Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Invest Ophthalmol Vis Sci 40:2442–2448
54. Hillard CJ (2008) Role of cannabinoids and endocannabinoids in cerebral ischemia. Curr Pharm Des 14:2347–2361
55. Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, Pizzolato G, Di M, V (2010) Possible anandamide and palmitoylethanolamide involvement in human stroke. Lipids Health Dis 9:47
56. Ternianov A, Perez-Ortiz JM, Solesio ME, Garcia-Gutierrez MS, Ortega-Alvaro A, Navarrete F, Leiva C, Galindo MF et al (2012) Overexpression of CB2 cannabinoid receptors results in neuroprotection against behavioral and neurochemical alterations induced by intracaudate administration of 6-hydroxydopamine. Neurobiol Aging 33:421–416
57. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di M, V, Doherty P (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163:463–468
58. Pasquare SJ, Giusto NM (1993) Differential properties of phosphatidate phosphohydrolase and diacylglyceride lipase activities in retinal subcellular fractions and rod outer segments. Comp Biochem Physiol B 104:141–148
59. Pasquare SJ, Salvador GA, Giusto NM (2008) Involvement of lysophosphatidic acid, sphingosine 1-phosphate and ceramide 1-phosphate in the metabolization of phosphatidic acid by lipid phosphate phosphatases in bovine rod outer segments. Neurochem Res 33:1205–1215
60. Pasquare SJ, Giusto NM (2008) Diacylglyceride lipase activity in rod outer segments depends on the illumination state of the retina. Neurochem Int 53:382–388
61. Pagano RE, Longmuir KJ (1985) Phosphorylation, transbilayer movement, and facilitated intracellular transport of diacylglycerol are involved in the uptake of a fluorescent analog of phosphatidic acid by cultured fibroblasts. J Biol Chem 260:1909–1916
62. Li J, Dong Y, Lu X, Wang L, Peng W, Zhang XC, Rao Z (2013) Crystal structures and biochemical studies of human lysophosphatidic acid phosphatase type 6. Protein Cell 4:548–561
63. Nevalainen T, Irving AJ (2010) GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? Curr Top Med Chem 10: 799–813
64. Labar G, Bauvois C, Borel F, Ferrer JL, Wouters J, Lambert DM (2010) Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. Chembiochem 11:218–227
65. Ghalayini AJ, Anderson RE (1992) Activation of bovine rod outer segment phospholipase C by arrestin. J Biol Chem 267:17977–17982
66. Kerov V, Chen D, Moussaif M, Chen YJ, Chen CK, Artemyev NO (2005) Transducin activation state controls its light-dependent translocation in rod photoreceptors. J Biol Chem 280:41069–41076
67. Strissel KJ, Lishko PV, Trieu LH, Kennedy MJ, Hurley JB, Arshavsky VY (2005) Recoverin undergoes light-dependent intracellular translocation in rod photoreceptors. J Biol Chem 280: 29250–29255
68. Deretic D, Williams AH, Ransom N, Morel V, Hargrave PA, Arendt A (2005) Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). Proc Natl Acad Sci U S A 102:3301–3306
69. Deretic D (2006) A role for rhodopsin in a signal transduction cascade that regulates membrane trafficking and photoreceptor polarity. Vis Res 46:4427–4433
70. Insinna C, Besharse JC (2008) Intraflagellar transport and the sensory outer segment of vertebrate photoreceptors. Dev Dyn 237: 1982–1992
71. Huttl S, Michalakis S, Seeliger M, Luo DG, Acar N, Geiger H, Hudl K, Mader R et al (2005) Impaired channel targeting and retinal degeneration in mice lacking the cyclic nucleotide-gated channel subunit CNGB1. J Neurosci 25:130–138
72. Tam BM, Moritz OL, Papermaster DS (2004) The C terminus of peripherin/rds participates in rod outer segment targeting and alignment of disk incisures. Mol Biol Cell 15:2027–2037
73. Pearring JN, Salinas RY, Baker SA, Arshavsky VY (2013) Protein sorting, targeting and trafficking in photoreceptor cells. Prog Retin Eye Res 36:24–51
74. Bouskila J, Harrar V, Javadi P, Beierschmitt A, Palmour R, Casanova C, Bouchard JF, Ptito M (2016) Cannabinoid receptors CB1 and CB2 modulate the electroretinographic waves in vervet monkeys. Neural Plast 2016:1253245
75. Chen Y, Luo X, Liu S, Shen Y (2018) Neuroprotective effect of cannabinoid receptor 1 antagonist in the MNU-induced retinal degeneration model. Exp Eye Res 167:145–151
76. Imamura T, Tsuruma K, Inoue Y, Otsuka T, Ohno Y, Ogami S, Yamane S, Shimazawa M et al (2018) Involvement of cannabinoid receptor type 2 in light-induced degeneration of cells from mouse retinal cell line in vitro and mouse photoreceptors in vivo. Exp Eye Res 167:44–50
77. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T (2007) Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun 362:928–934
78. Alhouayek M, Masquelier J, Muccioli GG (2018) Lysophosphatidylinositols, from cell membrane constituents to GPR55 ligands. Trends Pharmacol Sci 39:586–604
79. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di M, V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457
80. Bouskila J, Harrar V, Javadi P, Casanova C, Hirabayashi Y, Matsuo I, Ohyama J, Bouchard JF et al (2016) Scotopic vision in the monkey is modulated by the G protein-coupled receptor 55. Vis Neurosci 33:E006