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Review

CB_1R, CB_2R and TRPV1 expression and modulation in in vivo, animal glaucoma models: A systematic review

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ABSTRACT

Background: The endocannabinoid system (ECS) is a complex biological regulatory system. Its expression and functionality have been widely investigated in ocular tissues. Recent data have reported its modulation to be valid in determining an ocular hypotensive and a neuroprotective effect in preclinical animal models of glaucoma.

Aim: This study aimed to explore the available literature on cannabinoid receptor 1 (CB_1R), cannabinoid receptor 2 (CB_2R), and transient receptor potential vanilloid 1 (TRPV1) expression in the trabecular meshwork (TM), ciliary body (CB), and retina as well as their ocular hypotensive and neuroprotective effects in preclinical, in vivo, animal glaucoma models.

Materials and methods: The study adhered to both PRISMA and SYRCLE guidelines. Sixty-nine full-length articles were included in the final analysis.

Results: Preclinical studies indicated a widespread distribution of CB_1R, CB_2R, and TRPV1 in the TM, CB, and retina, although receptor-, age-, and species-dependent differences were observed. CB_1R and CB_2R modulation have been shown to exert ocular hypotensive effects in preclinical models via the regulation of inflow and outflow pathways. Retinal cell neuroprotection has been achieved in several experimental models, mediated by agonists and antagonists of CB_1R, CB_2R, and TRPV1.

Discussion: Despite the growing body of preclinical data regarding the expression and modulation of ECS in ocular tissues, the mechanisms responsible for the hypotensive and neuroprotective efficacy exerted by this system remain largely elusive. Research on this topic is advocated to further substantiate the hypothesis that the ECS is a new potential therapeutic target in the context of glaucoma.

1. Introduction

Glaucoma refers to a spectrum of optic neuropathies defined by the progressive degeneration of retinal ganglion cells (RGCs) and the coherent loss of RGC axons [1]. As a result, optic disc cupping, thinning of the inner retinal layers, and progressive visual field loss were observed [1]. While the pathogenic core of the disease is yet to be fully understood, several risk factors (i.e. genetic predisposition [2], age, smoking [3], hypercholesterolaemia [4], and high intraocular pressure (IOP) [1]) have been shown to contribute to the onset and progression of glaucoma [1].

Coherently, different approaches have been explored to hinder the natural course of the disease, with IOP-reducing strategies and neuroprotection being the most investigated [5]. Specifically, the term neuroprotection refers to non-IOP-related interventions that can delay or stop the progression of glaucomatous damage in affected individuals [6].

The endocannabinoid system (ECS) is a composite biological

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network that relies on the crosstalk between several regulatory enzymes (i.e. fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), soluble lipid mediators (i.e. anandamide (AEA), 2-arachidynoil-glycerol (2-AG)), specific cannabinoid (i.e. cannabinoid receptor 1 (CB_1R) and cannabinoid receptor 2 (CB_2R)), and non-cannabinoid (i.e. transient receptor potential vanilloid-1 (TRPV1)) receptors [7,8]. The expression of mediators and receptors has been variably demonstrated in nearly all human organs, where they play diverse tissue-specific functional roles [8–10].

From an ophthalmological point of view, while the ECS presents a widespread distribution in both the anterior and posterior segments of the eye, increasing number of evidence is available on the benefits derived from its modulation in different pathological conditions [9–11]. For example, from Hepler and Frank's seminal observation that inhaled, injected, or orally administered cannabinoids can reduce IOP, the ECS has been thoroughly analysed as a new potential therapeutic strategy to include in the armament of glaucoma specialists [12]. However, no cannabinoid drugs have been approved for glaucoma treatment.

Given the complexity of the ECS, the often overtly conflicting reported results, and the large volume of available data, we have found it useful to summarise the body of knowledge that has been added to the field in the last 20 years.

This review aimed to provide an updated overview of the evidence introduced in the last 20 years regarding the expression of CB_1R, CB_2R, and TRPV1 in the trabecular meshwork (TM), ciliary body (CB), and retina, as well as their ocular hypotensive and neuroprotective effects in preclinical, in vivo, glaucoma models.

2. Materials and methods

This review was compiled based on the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) and the 2020 updated Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines [13]. Since all data were obtained from the published literature, institutional review board approval and informed consent were not required for this study.

2.1. Eligibility criteria

No study protocol was registered for this review, which was conducted according to the modified PICOS acronym for animal studies (AICOS): animal (A), in vivo studies on different animal models of glaucoma; intervention (I), not required; control (C), not required; outcome (O), expression of the aforementioned receptor(s), hypotensive and neuroprotective efficacy; study design (S), preclinical, in vivo studies. Studies were excluded if they (a) were not in English, (b) were ongoing projects, (c) the article type was a review, a case report, a letter to the editor or a book chapter, (d) were published before January 01, 2000, (e) were analysed in vitro or on human subjects.

2.2. Data source and study searching

A review of the Medline (PubMed) and EMBASE libraries was performed. In the online supplementary materials, the search query applied to PubMed is available. We added a filter for animal studies proposed by Hooijmans et al. and suggested by the SYRCLE [14].

All results were merged, and duplicates were removed using the reference management software EndNote X9 (version X9.3.3). Two authors independently screened the list of unresolved articles. Abstracts were examined, and whenever appropriate, full texts were subjected to subsequent evaluations for eligibility. A snowballing method was applied to retrieve further papers from the reference lists of the sorted texts. A third reviewer was consulted to resolve any discrepancies.

3. Results

The initial search yielded 895 peer-reviewed articles. Following the removal of duplicates and initial screening of titles and abstracts, we evaluated 162 full-text articles. Only 69 studies were included in this review.

3.1. Cannabinoid receptor 1: expression in animal ocular tissues

CB_1R expression has been variably demonstrated in nearly all portions of animal eyes, as shown (Table 1) (Fig. 1).

The porcine eye (i.e. CB, iris, retina, choroid) has been described in two separate works by Stamer et al. and Njie et al. as being rich in CB_1R in the TM and in the CB cells at both the gene and protein levels [15,16].

In Vervet monkeys, Bouskila et al. pointed out that CB_1R tends to have widespread retinal localisation from the foveal pit to the far periphery and is expressed in the photoreceptor, outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cell layer (GCL) [17,18].

Working on the goldfish retina, Yazoulla et al demonstrated the expression of CB_1R by the photoreceptors (i.e., both cone and rods), the horizontal cells, the bipolar cells, the amacrine cells and the ganglion cells, both in the intracellular compartment and on the cell membrane surface. [19].

In addition, CB_1R expression in the animal retina is evident both in the pre- and post-natal states, and it appears to be modulated in a time-dependent fashion, as proposed by Zabouri et al., Begbie et al., and Silva Sampaio et al. in goldfish, rats, and chicks [20–22].

In addition, Maccarone et al. demonstrated CB1R localisation in both the OPL and IPL in rats and that CB_1R expression was modulated by ambient light variation [23].

CB_1R immunoreactivity has been anatomically and functionally demonstrated in *Xenopus laevis* tadpole retina by Miraucourt et al., who also proved that agonism of CB_1R is responsible for the increased contrast sensitivity under mesopic conditions [24] Aguirre et al. demonstrated that CB_1R expression in the bovine outer retina is dependent on light exposure. [25].

3.2. Cannabinoid receptor 1: IOP-lowering effect in animal ocular tissues

The IOP-modulating efficacy of CB_1R has been widely explored; however, only scarce and contradictory data are available regarding the specific physiological mechanisms underlying its IOP-lowering effect (Fig. 2).

In the porcine anterior segment perfused organ culture model, AEA (a partial agonist of CB_1R and a weak partial agonist/antagonist of CB_2R) was shown to induce a hypotensive effect primarily mediated by CB_1R [26].

In this regard, Laine et al. reported that the cellular uptake inhibition of AEA mediated by the administration of AM404 or olvanil decreased IOP in normotensive rabbits [27] In a different study, they also reported that using an AEA-degrading enzyme inhibitory agent (i.e. phenylmethylsulfonyl fluoride), which increases the local AEA concentration, favoured the CB_1R-dependent IOP-lowering effect of anandamide in Dutch belted rabbits [28].

In addition, Njie et al. reported that the local application of 2-AG, the main endogenous agonist for both CB_1R and CB_2R, caused a transient enhancement of aqueous humour outflow at 1 h after treatment in both a CB_1R- and CB_2R-dependent fashion, in a porcine anterior segment perfusion model [29].

Comparably, Miller et al. proved that 2-AG can determine the reduction of IOP in a concentration-, CB_1R-, and not CB_2R-dependent manner in C57BL/6J mice [30] In addition, they evaluated the 2-AG IOP-lowering efficacy in mice $CB_1R^{-/-}$ and $CB_2R^{-/-}$mice. While no variation in IOP profile was observed in $CB_1R^{-/-}$ mice, 2-AG was still effective in $CB_2R^{-/-}$ mice [30].

Table 1
CB_1R expression in animal trabecular meshwork, ciliary body and retina.

Author	Year	Species	Finding	mRNA	Protein	RoB
Yazulla et al. [19]	2000	*Carassius auratus*	CB_1R expression (protein level) in cytoplasm and pre-synaptic terminals of photoreceptors, amacrine cells, bipolar cells and Müller cells	NA	WB: single band at 70 kDa in retina. IHC: immunolabelling of the entire retina from the cell bodies of the cones to the vitreal border.	Low
Stamer et al. [15]	2001	Bovine	CB_1R expression (gene [in situ *hybridisation*] and protein level [IHC]) in trabecular meshwork and ciliary body	RT-PCR: 30 – 40 cycles to detect CB_1R in trabecular meshwork or ciliary process cells.	IF: specific staining of non-pigmented ciliary epithelial cells and trabecular mashwork cells.	Low
Begbie et al. [21]	2004	Chick embryo	Variable expression of CB_1R (protein level) during different stage of development	NA	*In situ* hybridisation and whole mount IHC which shows the distribution pattern of the CB_1R at various step of embryo development.	Low
Njie et al. [16]	2006	Porcine	CB_1R expression (protein level) in trabecular meshwork cells	NA	WB: single band of 64 kDa in trabecular meshwork cells. IHC: staining of the trabecular meshwork cells.	Low
Nucci et al. [50]	2007	Sprague-Dawley rat	CB_1R expression (protein level) in retina	NA	ELISA: CB_1R expression in retina. Radioligand binding studies reveal functional CB_1R in retina.	Low
Zabouri et al. [20]	2011	Long Evans rat	Variable expression of CB_1R (protein level) during different stage of development	NA	WB and IHC showing the presence and the distribution pattern of the CB_1R in the retina of young and adult subjects.	Low
Hudson et al. [38]	2011	C57BL/6 mice	CB_1R expression (protein level) in the epithelium of cyliary body and angle.	NA	IF: labelling for CB_1R in the anterior segment of the eye.	Low
Bouskila et al. [18]	2012	Vervet Monkey	CB_1R expression (protein level) in cone pedicles, bipolar cells, horizontal cells, amacrince cells, RGCs soma and axon	NA	WB: single band at 60 kDa in retina. IHC: labelling for CB_1R throughout the retina, decreasing with retinal eccentricity.	Low
Cecyre et al. [48]	2013	C57BL/6N and C57BL/6J mice	CB_1R expression (protein level) in the outer and inner segments and in the cell body of cones and rods, in amacrine, bipolar and retinal ganglion cells	NA	WB: CB_1R presence demonstrated in CB_2R *knock out* mice. IHC: diffuse staining of the retina	Low
Kokona et al. [52]	2015	Sprague-Dawley rat	CB_1R expression (gene and protein level)	RT-PCR: expression of CB_1R mRNA in retina	Radioligand binding studies reveal functional CB_1R in retina.	Low
Bouskila et al. [17]	2016	C57BL/6 mice, *Tupaia belangeri*, *Macaca mulatta, Chlorocebus sabaeus*	CB_1R expression (protein level) in all 10 retinal layers	NA	IF: strong labelling for CB_1R in the GCL and RNFL in all species.	Low
Maccarrone et al. [23]	2016	*Rattus norvegicus*	CB_1R epression (gene and protein level) in the OPL and IPL, dependent on light exposure	RT-PCR: expression of CB_1R mRNA in retina dependent upon light exposure.	WB: single band at 60 kDa in retina. IHC: CB_1R epression in the OPL and IPL.	Low
Miracourt et al. [24]	2016	*Xenopus laevis* tadpole	CB_1R epression (protein level) in the OPL and IPL	NA	IHC: intense labelling for CB_1R in the OPL and IPL	Low
da Silva Sampaio et al. [22]	2018	*Gallus gallus* embryo	Variable expression of CB_1R (protein level) during different stage of development	NA	WB: single band at 60 kDa in retina. IF: intense age-dependent retinal labelling.	Low
Miller et al. [42]	2018	C57BL/6 mice	CB_1R expression (gene level) in the eye is gender-related.	RT-PCR: expression of CB_1R mRNA in the eye is higher in male than in female subjects.	NA	Low
Aguirre et al. [25]	2019	Bovine	Expression of CB_1R (protein level) in the rod outer segment	NA	WB: single band at 45 kDa in retina.	Low
Kokona et al. [53]	2021	Sprague-Dawley rat	Expression of CB_1R (gene level) in the retina	RT-PCR: expression of CB_1R mRNA in the eye is higher than that of CB_2R	NA	Low

List of abbreviations. RoB: risk of bias; CB_1R: cannabinoid receptor 1; RGC: retinal ganglion cell; OPL: outer plexiform layer; IPL: inner plexiform layer; RT-PCR reverse trancriptase-polymerase chain reaction; WB: western blot; IHC: immunohistochemistry; IF: immunofluoresence.

A study by Laine et al. showed that 2-AG provide an IOP-lowering effect not directly mediated by CB_1R but through its prostanoid metabolites when topically applied in normotensive pigmented Dutch belted rabbits or New Zealand White albino rabbits, [31] This data was corroborated by the evidence of a null effect of AM251 (i.e. a CB_1R antagonist) on the IOP-lowering profile of 2-AG.

Song et al. ascertained that the topical application of WIN55212-2 (i.e. a full CB_1R agonist) significantly reduced intraocular pressure in the eyes of New Zealand White rabbits in a time- and dose-dependent manner [32]. Notably, no crossover hypotensive effect was observed in the contralateral control eye, and the IOP-lowering effect of WIN55212-2 was prevented by pretreatment with SR141716A, a CB_1R antagonist [32].

Similar results were recorded by Oltmanns et al. and Hosseini et al. using Sprague Dawley rats [33,34]. Hosseini's group extended the observation period up to 4 weeks. They also evaluated both the sustained and effective hypotensive effects of WIN55212-2 and the absence of systemic or local adverse events. [34].

Similarly, Chien et al., working on normotensive and glaucoma-induced *Macaca cynomolgus* monkeys, described a strong, sustained, and dose-dependent hypotensive effect of WIN55212–2 in the absence of topical side effects [35].

Recently, Miller et al. suggested that the topical instillation of a novel CB_1R agonist (i.e. AM7410) reduced IOP by up to 30% at 5 h post-administration in a C57BL/6J mouse strain. [36] They also demonstrated that the IOP-lowering effect of AM7410 was CB_1R-dependent, as

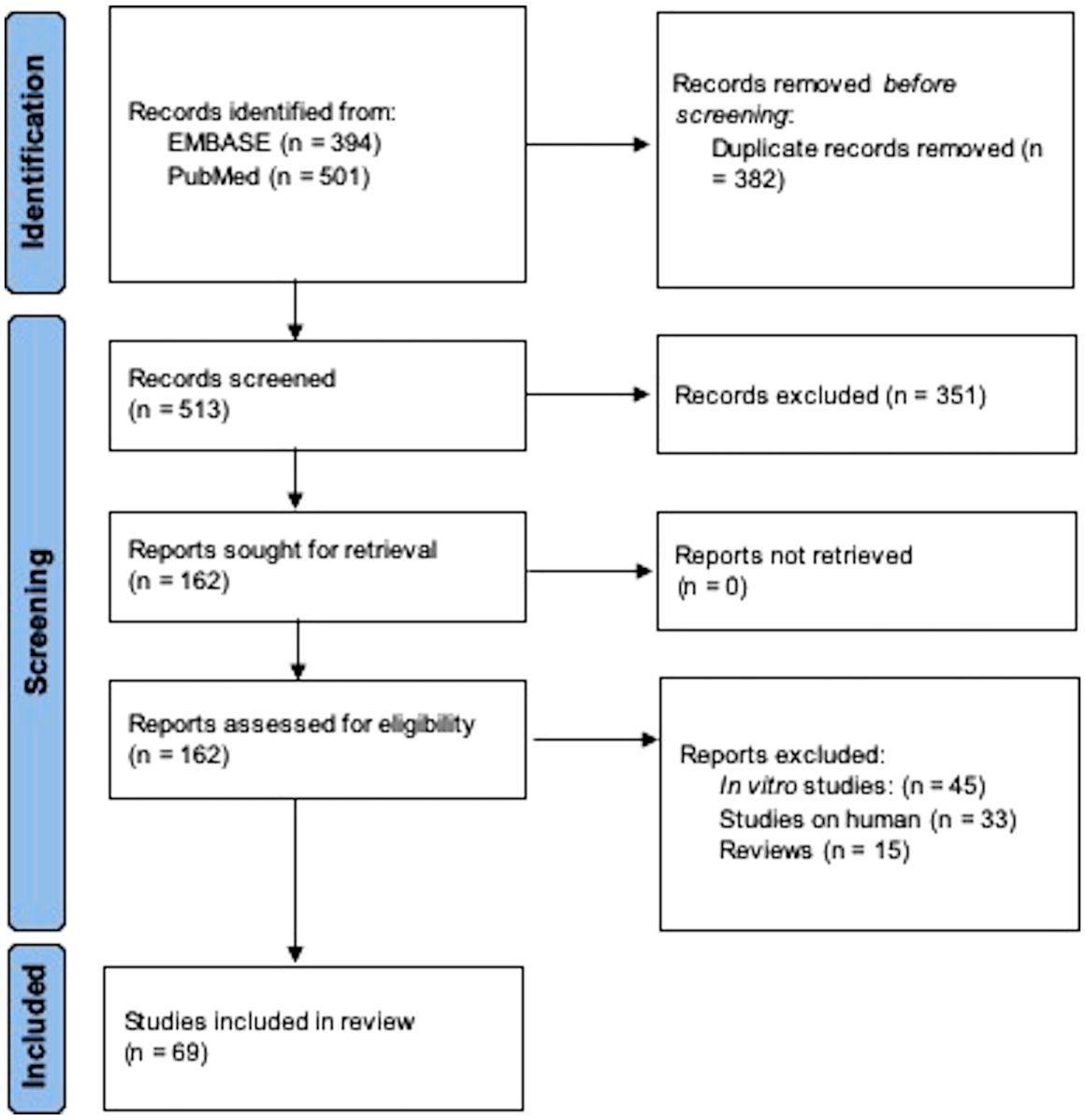

Fig. 1. PRISMA 2020 flow diagram mapping the number of retrieved, analysed, excluded, and included records.

it was not observed in CB_1R knockout mouse strains. [36].

In addition, it must be considered that Samudre et al. found that intravenous (IV)-injected WIN55212–2 possessed a higher IOP-lowering efficacy than the topical administration in New Zealand White rabbits. [37] Notably, while topical WIN55212–2 was able to exert a clinically relevant ocular hypotensive activity (i.e. 20% IOP reduction after single instillation, comparable to that induced by timolol eye drops), side effects (i.e. mainly bradycardia) were associated with the IV injection route. [37].

Hudson et al. discussed that the hypotensive activity of WIN55212–2 in mice depends, among others, on the presence of both adrenergic receptors and catecholamines [38,39].

The modulation of CB_1R by positive and negative allosteric modulators (i.e. positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs), respectively) has been proven to be effective in reducing IOP values in experimental animal models of ocular hypertension. For example, Cairns et al. showed that the use of PAM GAT229 decreased IOP in ocular hypertensive mice [40]. In addition, enhanced CB_1R-mediated IOP reduction was observed when GAT229 administration was combined with subthreshold doses of CB_1R orthosteric ligands in normotensive mice [40].

In addition, Miller et al. proved that both topical and IV application of two different CB_1R NAMs (i.e. ABD1085 and PSNCBAM1, respectively) yielded a significant drop in IOP in both normotensive and hypertensive mice [41].

However, CB_1R agonism by the well-known phytocannabinoid Δ(9)-THC or dronabinol has been shown to significantly reduce IOP for at least 8 h after topical application in C57BL/6J normotensive mice [42].

Fischer et al. consistently determined that twice-daily administration of a topically applied 2% THC ophthalmic solution resulted in a moderate IOP reduction in normal dogs without affecting the aqueous humour outflow rate [43].

The relatively hydrophilic THC prodrug, the amino acid (valine)-dicarboxylic acid (hemisuccinate) ester THC-Val-HS, showed a slightly better IOP-lowering effect than timolol, with the former having a shorter duration of action, in the α-chymotrypsin induced rabbit glaucoma model [44]. In addition, when the hypotensive efficacy of THC-Val-HS was compared with another marketed antiglaucoma product, pilocarpine eye drops showed better IOP-lowering activity [44].

Moreover, the ocular hypotensive effect of THC-Val-HS was higher

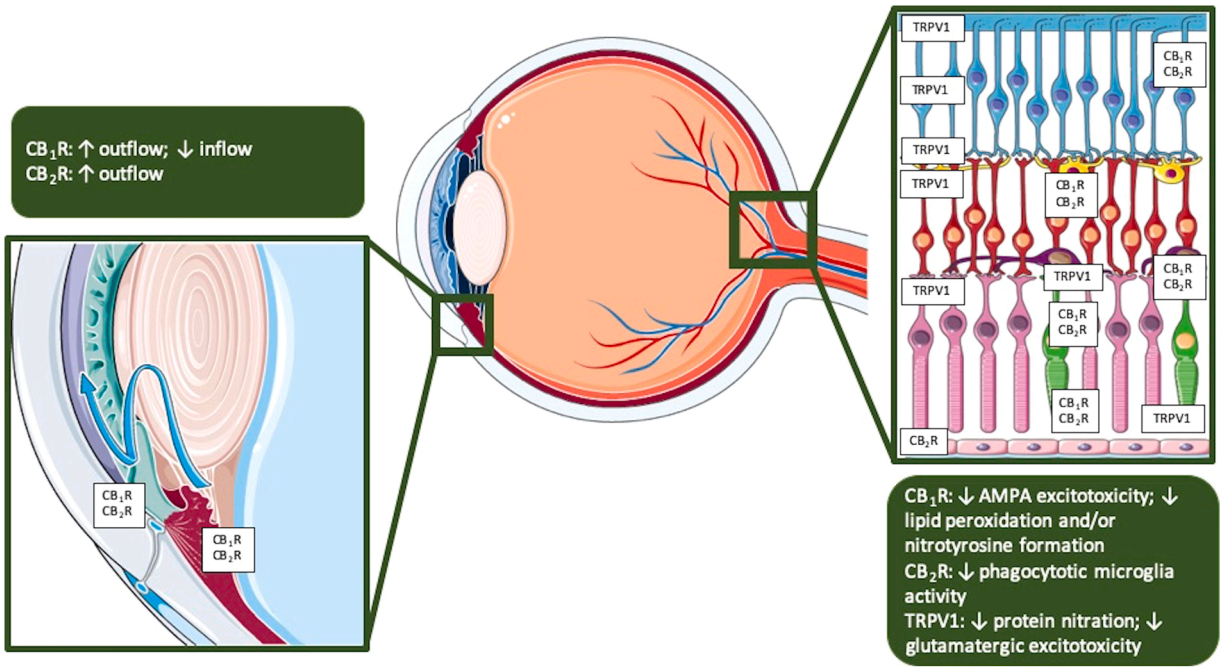

Fig. 2. Localisation of CB_1R, CB_2R, and TRPV1 in the trabecular meshwork, ciliary body, and retina has been proposed in the current literature. While CB_1R and CB_2R seem to be widely expressed in the eye, TRPV1 appears to localise only in neural tissues (i.e. the retina). In squares, hypothesised hypotensive and neuroprotective mechanisms are promoted by these three receptors. (CB_1R, cannabinoid receptor 1; CB_2R, cannabinoid receptor 2; TRPV1, transient receptor potential vanilloid 1).

when the prodrug was administered in the form of a solid lipid nanoparticle than when it was administered in a normotensive rabbit model [45] In this case, THC-Val-HS was reported to be a more effective IOP-reducing agent than timolol maleate and pilocarpine [45].

Even liposomal formulations of intratracheally administered dronabinol provided CB_1R-dependent IOP-lowering efficacy in Brown Norway rats, as shown by Szczesniak et al. in 2005 [46].

3.3. Cannabinoid receptor 1: neuroprotective effect in animal ocular tissues

CB_1R modulation in animal experimental models has been proven to harness IOP-lowering and neuroprotective effects (Table 2) (Fig. 2).

As a proof of concept, the use of a CB1R antagonist increased the amplitude of the a-wave at high flash intensity values in healthy vervet monkeys [47]. However, under scotopic conditions, CB_1R was shown to increase only the amplitude of the b-wave, irrespective of the flash intensity [47].

Cécyre et al. showed that the absence of CB_1R in knockout mice does not affect the electroretinogram (ERG) response [48]. However, they suggested that the absence of functional changes might derive from a low detection sensitivity of the ERG measurement rather than from a null effect of CB_1R on neuroretinal cell functionality [48].

These data demonstrate that CB_1R localises and modulates retinal cell functionality.

Topical administration of WIN55212–2 has been shown to exert a neuroprotective effect on RGCs in a high-pressure-induced-ischaemia reperfusion model in Sprague Dawley rats [49].

In the same glaucoma model, our group demonstrated that a high IOP-induced ischaemic insult, followed by reperfusion, results in a significant decrease in the endogenous tone of AEA, associated with an increased activity of FAAH and consequent RGCs loss [50]. Conversely, we reported that intravitreal injection of the stable AEA analogue, methanandamide (MetAEA), and systemic administration of the FAAH inhibitor, URB597, can prevent RGCs loss in a CB_1R- and TRPV1-dependent manner [50].

Similarly, Slusar et al. assessed that the FAAH inhibitor URB597 promotes retinal ganglion cell neuroprotection in a rat model of optic nerve axotomy [51]. The inhibition of FAAH favours an increase in the local AEA concentration, thus promoting CB_1R-dependent neuroprotective action [51]. This neuroprotective effect was more evident in young than in aged rats, which might promote the concept of age-dependent downregulation of the ECS in the retina [51].

Kokona et al., in a rat model of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) excitotoxicity, reported that endogenous cannabinoid AEA and synthetic cannabinoids HU-210 and MethAEA can prevent RGCs apoptosis via a CB_1R-dependent mechanism [52]. In addition, they highlighted that the PI3K/Akt and/or MEK/ERK1/2 signalling pathways are involved in the anti-apoptotic CB_1R intracellular cascade [52]. In a recent study, the same group determined that 2-AG was able to express similar neuroprotective efficacy in a CB_1R- and CB_2R-dependent fashion, both activating the PI3K signalling cascade and hampering microglial reactivity [53].

Similarly, in 2007, Crandall et al. elucidated the THC-mediated neuroprotective efficacy of CB1R in a rat model of cauterised-episcleral vein glaucoma [54]. They showed that weekly intraperitoneal (IP) injections of THC were able to prevent RGCs loss by up to 40% [54].

Using the same glaucoma model, El-Remessy et al. determined that THC exerts a neuroprotective effect on RGCs by attenuating lipid peroxidation and/or nitrotyrosine formation in a CB_1R- and dose-dependent manner [55].

3.4. Cannabinoid receptor 2: expression in animal ocular tissues

The expression of CB_2R in the TM and CB remains controversial (Table 3) (Fig. 2).

He et al. and Zhong et al. provided evidence of CB_2R immunoreactivity and functionality in the TM in a porcine perfusion anterior segment model [56,57]. However, no data on this topic have been provided so far.

Using in situ hybridisation, Lu et al. demonstrated strong labelling of

Table 2
Evidence on the neuroprotective efficacy of CB_1R modulation.

Author	Year	Species	Finding	RoB
El-Remessey et al. [55]	2003	*Rattus norvegicus*	CB_1R agonism by THC and CBD is able to prevent RGC loss	Low
Crandall et al. [54]	2007	*Rattus norvegicus*	CB_1R agonism by THC is able to prevent RGC loss	Moderate
Nucci et al. [50]	2007	*Rattus norvegicus*	CB_1R agonism is able to prevent RGC loss	Low
Slusar et al. [51]	2013	Fischer-344 rats	CB_1R agonism hampers microglial activation and prevent RGC loss	Low
Cecyre et al. [48]	2013	C57BL/6N and C57BL/6J mice	Deletion of CB_1R does not modify ERG recordings	Low
Pinar-Sueiro et al. [49]	2013	*Rattus norvegicus*	CB_1R agonism prevent RGC loss in a IOP-independent fashion	Low
Kokona et al. [52]	2015	*Rattus norvegicus*	CB_1R agonism prevent RGC loss via mechanisms involving the PI3K/Akt and/or MEK/ERK1/2 signalling pathways	Low
Maccarrone et al. [23]	2016	*Rattus norvegicus*	CB_1R antagonism protects photoreceptors from light-induced neurodegeneration	Low
Bouskila et al. [47]	2016	*Chlorocebus sabaeus*	Blockade of CB_1R variably modulates the ERG recordings	Low
Cecyre et al. [65]	2020	C57BL/6N and C57BL/6J mice	Deletion or blockade of CB_1R does not modulate visual acuity	Low
Kokona et al. [53]	2021	*Rattus norvegicus* and C57BL/6 mice	CB_1R agonism attenuates RGC loss via the activation of the PI3K/Akt downstream signalling pathway and modulation of the glia activation	Low

List of abbreviations. RoB: risk of bias; CB_1R: cannabinoid receptor 1; THC: Δ(9)-tetrahydrocannabinol; CBD: cannabidiol; RGC: retinal ganglion cell; ERG: electroretinogram; IOP: intraocular pressure.

CB_2R mRNA in the GCL, IPL, and inner photoreceptor segment layer of the rat retina [58]. However, Porcella et al., did not detect CB_2R mRNA in the rat retina using different transcripts [59]. In contrast, this last piece of evidence must be cautiously handled, as per the moderate RoB of the publication (Table 3). According to the author, data regarding the null expression of CB_2R transcripts are not shown in the manuscript [59].

In wild-type mice, Cécyre et al. showed CB_2R immunoreactivity in cone and rod photoreceptors, horizontal cells, some amacrine cells, bipolar cells, and ganglion cells [48].

A study by Lopez et al. showed that CB_2R localises to the retinal pigmentary epithelium, inner photoreceptor segments, INL, IPL, and GCL [60]. No double labelling with specific retinal cell markers was performed in this study, and different cell types were identified based on the topographical and morphological data [60].

Using immunohistochemistry, Maccarrone et al. demonstrated that CB_2R is expressed in the inner segment of photoreceptor cells and the inner retina (INL and GCL) of Sprague Dawley and albino rats [23]. In addition, they showed that CB_2R expression is strictly dependent on environmental stimuli, such as continuous bright light exposure and saffron supplementation [23]. Similar results were obtained by Aguirre et al. in the bovine retina [25].

In a recently study, Borowska-Fielding et al. proposed that CB_2R is expressed in the retina of C57/BL6 mice only in the OPL, IPL, and GCL [61].

The discrepancies among the aforementioned results might be explained by the strict functional and anatomical relationship between Müller cell glia and neuroretinal cells. Bouskila et al. demonstrated that CB_2R is present in the retina of vervet monkeys, specifically in retinal Müller cells [62]. Hence, it cannot be excluded that the expression of CB_2R in the GCL, as reported by different groups, might be derived from the inclusion of Müller cell processes in the tissue samples used.

In addition, it must be considered that different techniques have been used over time to ascertain the retinal expression of CB_2R (i.e. IF, WB, and in situ hybridisation), which often results in conflicting results [48,58,60].

Hence, although CB_2R expression in retinal tissues cannot be excluded, its exact cellular and topographical expression patterns remain elusive.

3.5. Cannabinoid receptor 2: IOP-lowering effect in animal ocular tissues

Using immunofluorescence microscopy and western blot analysis, He et al. and Zhong et al. supported the hypothesis that CB_2R agonism might increase aqueous humour outflow (Fig. 2) [56,57]. In two different studies, they proved that the application of the synthetic full CB_2R agonist, JWH015, was able to favour aqueous humour outflow, modulating the intracellular morphology of TM cells [56,57].

This hypothesis was further supported by the evidence provided by Njie et al. that 2-AG can improve the humour outflow facility [29]. In the same model, similar results were obtained with the administration of AEA, although the role played by CB_2R was shown to be only partial [26].

Furthermore, the CB_2R-dependent IOP-lowering effect might be mediated by palmitoylethanolamide (PEA) [63]. In a porcine model, Kumar et al. proved that PEA treatment can increase aqueous humour outflow in a CB_2R-dependent and CB_1R-independent fashion [63]. However, as PEA is not able to directly bind CB_2R, it might be postulated that its hypotensive efficacy is exerted by a receptor, in contrast to CB_1R and CB_2R, which in turn interacts with CB_2R [63].

These data appeared to be in contrast to those proposed by Laine et al. [64]. Working on normotensive rabbits, and determined that topical application of the CB_2R agonist, JWH-133, does not exert any hypotensive efficacy [64].

This discrepancy might be explained by differences in the experimental model. First, in Laine et al., CB_2R modulation was evaluated in an in vivo-*only* model. Although the use of $CB_2R^{-/-}$ by Hudson et al. [38] and Miller et al. [30] is a strong argument against the presumed CB_2R-hypotensive effect, it is possible that under certain conditions, CB_2R is upregulated and, therefore, able to act as an IOP modulator.

It should also be noted that some of the evidence for a CB_2R role relies on the ostensible CB_2R-selectivity of JWH015, a compound that can act as an efficacious CB1 agonist [30]. Hence, it cannot be excluded that some bias might have been derived from studies in which JWH015 was used in $CB_1R^{+/+}$/ $CB_2R^{+/+}$ animal models.

3.6. Cannabinoid receptor 2: neuroprotective effect in animal ocular tissues

The hypothesis of the functional involvement of CB_2R in retinal cells was demonstrated by Bouskila et al. in healthy vervet monkeys (Table 4) (Fig. 2) [47]. Following the blockade of CB_2R by the full antagonist AM630, the amplitude of the b-wave increased in photopic conditions. At high flash intensity values, blockage of CB_2R determined an increase in the a-wave, whereas, under scotopic conditions, CB_2R antagonism provided an increase in the b-wave only [47].

Borowska-Fielding et al. reported that prolonged blockage of CB_2R can alter retinal signalling, as evidenced by the enhancement of scotopic a-wave, dark-adapted cone-driven response, and photopic b-wave in ERG measurements [61]. These results were comparable to those obtained in $CB_2R^{-/-}$ mice [61].

Cécyre et al. highlighted that in $CB_2R^{-/-}$, the amplitudes of the ERG a-

Table 3
CB_2R expression in animal trabecular meshwork, ciliary body and retina.

Author	Year	Species	Finding	mRNA	Protein	RoB
Porcella* et al. [59]	2000	Rat	CB_2R expression (gene and protein level) not detected in any ocular structure	NA	NA	Moderate
Lu et al. [58]	2000	*Rattus norvegicus*	CB_2R expression (gene and protein level) in the inner photoreceptor segments, INL and GCL layer	RT-PCR: mRNA for CB_2R of around 560 base pairs in retina.	ISHH: presence of CB_2R mainly in inner retinal layers.	Low
Zhong et al. [57]	2005	Porcine	CB_2R expression (protein level) in trabecular meshwork cells	NA	IF: expression of CB_2R on trabecular meshwork cells	Low
He et al. [56]	2007	Porcine	CB_2R expression (protein level) in trabecular meshwork cells	NA	WB: single band at 44 kDa. IF: positive signals were detected on porcine trabecular meshwork cells.	Low
Lopez et al. [60]	2011	*Rattus norvegicus*	CB_2R expression (protein level) in retinal pigmentary epithelium, inner photoreceptor segments, IPL, and GCL	NA	IHC: strong labelling in all the retinal layers and in the RPE.	Low
Bouskila et al. [62]	2013	*Chlorocebus sabeus*	CB_2R expression (protein level) in Müller cells only	NA	WB: single band at 45 kDa. IHC: strong labelling in inner retinal layers.	Low
Cecyre et al. [48]	2013	C57BL/6N and C57BL/6J mice	CB2R expression (protein level) in the outer and inner segments and in the cell body of cones and rods, in amacrine, bipolar and retinal ganglion cells	NA	WB: CB_2R presence demonstrated in CB_1R *knock out* mice. IHC: diffuse staining of the retina	Low
Kokona et al. [52]	2015	Sprague-Dawley rat	CB_2R expression (gene and protein level)	RT-PCR: expression of CB_2R mRNA in retina	Radioligand binding studies reveal functional CB_2R in retina.	Low
Maccarrone et al. [23]	2016	*Rattus norvegicus*	CB_2R epression (protein level) in the inner segment of photoreceptors, INL and GCL	RT-PCR: expression of CB_2R mRNA in retina dependent upon light exposure.	WB: single band at 45 kDa in retina. IHC: CB_2R epression in the inner retina.	Low
Borowska-Fielding et al. [61]	2018	C57BL/6J mice	CB_2R expression (protein level) by a small subsets of microglial cells in the INL	NA	IHC: only minimal staining in mice retina, dependent upon the presence of inflammatory stimuli.	Low
Aguirre et al [25]	2019	Bovine	Expression of CB_1R (protein level) in the rod outer segment	NA	WB: double band at 45 kDa in retina.	Low
Kokona et al [53]	2021	Sprague-Dawley rat	Expression of CB_2R (gene level) in the retina	RT-PCR: expression of CB_2R mRNA approximately correspond at 62% of that of CB1R	NA	Low

* Data not shown.
List of abbreviations. RoB: risk of bias; CB_1R: cannabinoid receptor 1; RGC: retinal ganglion cell; OPL: outer plexiform layer; IPL: inner plexiform layer; RPE: retinal pigmented epithelium; RT-PCR reverse trancriptase-polymerase chain reaction; WB: western blot; IHC: immunohistochemistry; IF: immunofluoresence; ISHH: In situ hybridisation histochemistry.

wave were increased, resulting from a slower deceleration rather than an increase in the acceleration of the waveform [48]. In addition, under photopic conditions, the b-wave amplitudes in CB_2R knockout mice required more light adaptation time to reach stable values [48].

A recent study from the same group demonstrated that both deletion and blockage of CB_2R increased visual acuity, whereas no impact of CB_1R was detected using the optomotor response in mice [65]. However, no effect of CB_2R modulation was observed on the ERG recordings [65].

These data appear to promote CB_2R as merely expressed and responsible for the functional modulation of neuroretinal cells in animal tissues.

The major expression of CB_2R in Müller cell glia is responsible for the neuroprotective efficacy of CB2R agonists. Slusar et al., working on Fischer-344 rats, demonstrated increasing AEA levels via direct URB597-mediated inhibition of FAAH hampers microglial activation [51]. This effect is prevented by the CB_2R antagonist AM630, resulting in an increased phagocytic microglial activity [51].

The local expression of CB_2R appeared to be accelerated in the presence of continuous bright light exposure in Sprague Dawley and albino rats [23]. Notably, increased levels of CB_2R in this context appeared to be associated with progressively worsening inner retinal function, as demonstrated by the decreased amplitude of the flash electroretinogram b-wave. These effects appeared to be hampered by the downregulation of both CB_1R and CB_2R, as further demonstrated by measurements of damaged areas in the outer nuclear layer (ONL) [23].

The mismatch between the aforementioned results might be explained by different experimental settings. The neuroprotective efficacy of CB_2R antagonism in the study by Maccarone et al. appeared to be expressed in an age-related macular degeneration model rather than a glaucoma model [23]. Hence, it can be speculated that different environmental stimuli induce a multiphasic, specific adaptive response driven by the ECS.

3.7. Transient receptor potential vanilloid 1: expression in animal ocular tissues

TRPV1 expression in animal tissues, which has been widely debated, is currently believed to be prominent in the retina (Table 5) (Fig. 2).

However, Choi et al., working on different mouse strains (C57BL/6, DBA/2J, and DBA/2J Gpnmb+ as a non-glaucomatous control of the same genetic background as DBA/2J), did not detect TRPV1 mRNA in the optic nerve head using single-cell reverse transcriptase-polymerase chain reaction (RT-PCR) [66].

In contrast, other groups, using various detection methods (i.e. western blot, immunohistochemistry, fluorescent in situ hybridisation) reported the expression of TRPV1 in photoreceptors, bipolar, amacrine cells, and subsets of RGCs, as demonstrated in goldfish, zebrafish, mouse, rabbit, cat, and vervet monkey models [50,67–72]. Accordingly, in goldfish and zebrafish retinas, TRPV1 expression appears to be restricted to photoreceptor synaptic ribbons, whereas in amacrine cells, it has been shown to co-localise with FAAH [67,73].

Bouskila et al. showed that TRPV1 is mainly expressed in horizontal

Table 4
Evidence on the neuroprotective efficacy of CB_2R modulation.

Author	Year	Species	Finding	RoB
Slusar et al. [51]	2013	Fischer-344 rats	CB_2R agonism hampers microglial activation	Low
Cecyre et al. [48]	2013	C57BL/6N and C57BL/6J mice	Deletion of CB_2R increase amplitude of the ERG a-wave	Low
Maccarrone et al. [23]	2016	*Rattus norvegicus*	CB_2R antagonism protects photoreceptors from light-induced neurodegeneration	Low
Bouskila et al. [47]	2016	*Chlorocebus sabaeus*	Blockade of CB_2R variably modulates the ERG recordings	Low
Borowska-Fielding et al. [61]	2018	C57BL/6J mice	Deletion or blockade of CB_2R enhances scotopic a-wave, dark-adapted cone-driven response and photopic b-wave at the ERG measurements	Low
Cecyre et al. [65]	2020	C57BL/6N and C57BL/6J mice	Deletion or blockade of CB_2R increased the visual acuity, while the activation of CB2R decreased it.	Low
Kokona et al. [53]	2021	*Rattus norvegicus* and C57BL/6 mice	CB_2R agonism attenuates RGC loss via the activation of the PI3K/Akt downstream signalling pathway and modulation of the glia activation	Low

List of abbreviations. RoB: risk of bias; CB_2R: cannabinoid receptor 2; ERG: electroretinogram; RGC: retinal ganglion cell

inhibitory γ-aminobutyric acid (GABA)ergic cells that link photoreceptors and amacrines connecting bipolar cells [72]. In addition, Lakk et al. reported that approximately 40% of RGCs in the vertebrate retina express TRPV1, with only scarce data on the subcellular localisation of the receptor [68]. A study showed that a strong labelling for TRPV1 was identified in the perikarya of RGCs subsets, whereas Weitlauf et al. described an intense immunolabelling for TRPV1 both in the context of RGCs bodies and in the retinal nerve fibre layer (RNFL) where their axons extend [74,75]. Although apparently negligible, the low expression of Trpv1 is believed to be sufficient to support TRPV1 physiological function in RGCs [68].

TRPV1 expression has been shown to have both age- and pressure-dependent expression patterns [75]. Spatial and temporal variations in retinal TRPV1 distribution can be highlighted early in retinal development, as proposed by Leonelli et al. in embryo rats [69].

Furthermore, in DBA/2J mice, Weitlauf et al. and Sappington et al. determined that mRNA coding for TRPV1 has an RGCs concentration pattern higher in high- than in low-pressure cases [76–78]. In addition, old low- and high-IOP samples showed greater TRPV1 mRNA transcripts than their 3–5-month counterparts [75].

In contrast, we observed a progressive reduction in the expression of both TRPV1 and CB_1R in a rat model of high intraocular pressure-induced ischaemia [50].

This discrepancy might be explained by different experimental settings. While DBA/2J is a commonly accepted model of chronic primary open-angle glaucoma, IOP-induced retinal ischaemia more coherently represents a model of acute glaucoma.

Interestingly, TRPV1 has been shown to co-localise with TRPV4 and CB_1R in the same cell type [68,74]. Specifically, while contradictory data are available regarding any functional relationship existing between TRPV1 and TRPV4 [68,77], an inhibitory effect of CB_1R on TRPV1 has been described in RGCs of the murine retina by Jo et al. upon the agonism of ECS with both endogenous and synthetic ligands (i.e. 2-AG, WIN55,122-2) [74].

3.8. Transient receptor potential vanilloid 1: neuroprotective effect in animal ocular tissues

TRPV1 mostly acts as a mechanochemical sensor capable of inducing a net intracellular calcium influx (Table 6) (Fig. 2) [9]. In this context, as shown by Weitlauf et al., the transient increase in the local expression of TRPV1 induced by the hastened IOP determines the net hyperpolarising influence on ganglion cell firing rates [75,76]. As a proof of concept, they showed that $TRPV1^{-/-}$ RGCs completely lacked the compensatory transient increase in the number of TRPV1 receptors after the IOP increase, and they did not experience any appreciable increase in firing rates [75,76].

Similar results were reported by Sappington et al., who described a greater depolarising current driving a threshold rate in *Trpv1*$^{-/-}$ mouse retinas than in C57 mice [79]. However, they reported that *Trpv1*$^{-/-}$ accelerates optic nerve axonopathy with elevated IOP, reducing the nerve area, axon density, and axon transport to the brain [79].

The loss of the stress-related enhancement of RGCs excitability is counterintuitively accompanied by an increase in compound action potential, as reported by McGrady et al. [80]. Mechanistically, this evidence is explained by the rearrangements of the voltage-gated sodium channel NaV1.6 in the Ranvier nodes [80].

The net hyperpolarised state and the hastened electrical activity might result from the increase in the cytoplasmic calcium concentration, which is mediated by both extracellular influx and endoplasmic reticulum outflow, as demonstrated by Sappington et al. [78].

They showed that Ca^{2+} overload via TRPV1 can induce apoptosis in isolated RGCs [78]. However, in 2 different studies, Leonelli et al. showed that RGCs inactivation might be obtained only at very high concentrations of TRPV1 agonists (Table 7). [81,82] In addition, they demonstrated that RGCs death upon stress might depend on TRPV1 via two different mechanisms: hastening protein nitration mediated by the induction of nitric oxide synthase (NOS) and NMDA-mediated glutamatergic excitotoxicity. [81,82] This neuroprotective effect was demonstrated to be fully reversed by the administration of the TRPV1 antagonist capsazepine by Sakamoto et al. [83].

4. Discussion

To the best of our knowledge, this study represents the first systematic review to summarise the available data on CB_1R, CB_2R, and TRPV1 expression and modulation in pre-clinical animal glaucoma models, based on studies conducted in the last 20 years.

From the Hepler and Frank seminal incidental observation of an IOP-lowering effect of marijuana smoking in a subset of young male adults, research on this topic has flourished, attempting to provide a rationale for an eventual modulation of the ECS in the context of glaucoma [12,84]. However, as shown by our results, only conflicting evidence is available regarding both the exact topographical location and the functional role expressed by the ECS both in health and disease.

Specifically, while the immunoreactivity and functionality of CB_1R in TM and CB are strongly supported by the present data, only scarce information is currently available regarding TRPV1 and CB_2R expression in those sites [74]. However, as TRPV1 has been shown to localise and functionally interact with components of the autonomous nervous system in other organs, it cannot be excluded that such an interplay might exist even in the TM and CB, where autonomous nerve endings are represented [85,86].

In addition, data regarding CB_2R immunoreactivity in TM and CB derives from preliminary observations of the porcine perfused anterior segment model, but other pieces of evidence seem to prove otherwise [56,57,59].

In the animal retina, ECS components appear to have a broad distribution, with specific immunoreactivity for each of the analysed receptors. In fact, CB_1R and CB_2R have been detected in both the nuclear and synaptic layers, not only on the cellular surface but also in the

Table 5
TRPV1 expression in animal trabecular meshwork, ciliary body and retina.

Author	Year	Species	Finding			RoB
Zimov et al. [67]	2004	*Carassius auratus*	TRPV1 expression (protein level) diffuse in all the retina, majorly in the synaptic ribbons of photoreceptor cells	NA	IF: TRPV1 immunoreactivity detected in both inner and outer retinal regions	Low
Yazulla et al. [71]	2004	Albino rats, cats, *Macaca mulatta* and *Macaca fascicularis*	TRPV1 expression (protein level) in the IPL, INL and OPL	NA	IHC: major distribution of TRPV1 for all three species in the inner plexifoIPL) with fine very scattered process in the inner nuclear layer (INL) and outer plexiform layer	Moderate
Zimov et al. [73]	2007	*Carassius auratus, Brachydanio rerio*	TRPV1 expression (protein level) by amacrine cells	NA	WB: 3 different bands at 61, 196 and 227 kDa. IHC: very narrow region of the outer plexiform layer (OPL) of both goldfish and zebrafish.	Low
Nucci et al. [50]	2007	Sprague-Dawley rat	CB_1R expression (protein level) in retina	NA	ELISA: TRPV1 expression in retina. Radioligand binding studies reveal functional TRPV1 in retina.	Low
Leonelli et al. [69]	2009	*Rattusnorvegicus*	Variable expression of TRPV1 (gene and protein level) during different stage of development	RT-PCR: TRPV1 transcripts constantly detactable during different developmental stages.	IF: TRPV1 expression is stage-dependent and more prominent in inner nuclear layers of the retina.	Low
Sappington et al. [78]	2009	C57BL/6 J mice, DBA/2 J mice and *Rattus norvegicus*	TRPV1 expression (gene and protein level) in RGCs (dendrites, cells bodies and axons) and microglial cells	RT-PCR: TRPV1 transcripts present in retina and RGCs. FISH: TRPV1 transcripts in RGCs and microglia.	IHC: strong localisation in the outer retina and in RGCs. WB: single band at 100 kDa.	Low
Leonelli et al. [82]	2010	*Rattus norvegicus*	TRPV1 expression (protein level) in *naïve* and axonotomized rats	NA	WB: single band at 97 kDa. IHC: localised staining in the INL and GCL. IF: immunoreactivity in mainly localised around RGC bodies.	
Martinez-Garcia et al. [70]	2013	New Zealand White rabbits	TRPV1 expression (gene and protein level) in different part of the eye.	RT-PCR: TRPV1 transcripts detected in cornea, lens, ciliary body and retina.	IHC: uniform distribution of TRPV1throughout the retina, with the RPE showing the most intense staining. Significant TRPV1 staining was absent in ciliary processes	Low
Weitlauf et al. [76]	2013	C57BL/6J mice	TRPV1 expression (protein level) in the IPL	NA	IHC: TRPV1 localisation in the IPL is IOP-dependent	Moderate
Weitlauf et al. [75]	2014	C57BL/6J and DBA/2J mice	TRPV1 expression (gene and protein level) in RGCs bodies and in the IPL, depending on IOP levels	RT-PCR: TRPV1 transcripts quantity in retina is IOP- and age-dependent.	IF: TRPV1 localise primarily in the GCL and in the IPL.	Low
Choi et al. [66]	2015	C57BL/6J and DBA/2J mice	TRPV1 expression (gene level) not detected in the optic nerve head	RT-PCR: no transcripts detectable in the optic nerve head	NA	Low
Sappington et al. [77]	2015	C57BL/6J and DBA/2J mice, Sprague-Dawley rats and macaque monkeys	TRPV1 expression (gene and protein level) in RGCs	RT-PCR: TRPV1 transcripts in retina dependent upon IOP.	WB: single band at 100 kDa. IF: TRPV1 localise in GCL and colocalize with TRPV4 in specific RGC subsets.	Moderate
Jo et al. [74]	2017	Different mouse strains	TRPV1 expression (protein level) confined to a subset of RGCs	NA	IF: TRPV1 is present ina subset of RGCs where it colocalizes with CB1R.	Low
Lakk et al. [68]	2018	C57BL/6J mutant mice strain	TRPV1 expression (gene and protein level) by the SMI-32 + αRGCs	RT-PCR: TRPV1 transcripts (about 200 bp) detected at relatively low level in RGCs.	IF: TRPV1 is expressed by RGCs, cholocalizing with TRPV1 in specific cell subsets.	Low
Aguirre et al. [25]	2019	Bovine	Expression of CB_1R (protein level) in the rod outer segment	NA	WB: single band at 100 kDa in retina.	Low
Bouskila et al. [72]	2020	*Chlorocebus sabeus*	TRPV1 expression (protein level) by the horizontal, amacrine and RGCs.	NA	WB: single band at 100 kDa. IF: strong labelling for TRPV1 mostly pronounced in the GCL.	Low

List of abbreviations. RoB: risk of bias; TRPV1: transient receptor potential vanilloid 1; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; RGC: retinal ganglion cell; bp: base pairs; IOP: intraocular pressure; FISH: fluorescent in situ hybridisation; IIHC: immunohistochemistry; IF: immunofluoresence; WB: western blot.

intracellular compartment [60,87]. In contrast, TRPV1 seems to have a more selective localisation of photoreceptors, amacrine receptors, and RGCs [74,88].

As evidenced, wide heterogeneity emerged from the analysis of the obtained results. Therefore, the lack of specific antibodies for immunostaining and western blotting has been claimed to be the main reason for the discrepancies in the topic [89]. However, as shown in several studies, the expression pattern of ECS components appears to be modulated by a plethora of external stimuli, primarily inflammatory in nature [50,56,57]. Hence, the adoption of diverse experimental models could affect the reliability of results.

Notably, a relatively low expression of ECS components should be considered when analysing the literature [68]. It cannot be excluded that the *null* expression of the ECS constituents in a couple of reports might be derived from the low sensitivity threshold of the applied detection method. Finally, species-specific, topographic, and age-related expression of the ECS in different organs and tissues could not be excluded, which could affect both the extensibility and comparability of the available data [74,87].

The IOP-lowering profile derived from the modulation of the

Table 6
Evidence on the neuroprotective efficacy of TRPV1 modulation.

Author	Year	Species	Finding	RoB
Nucci et al. [50]	2007	*Rattus norvegicus*	TRPV1 agonism is able to prevent RGC loss	Low
Sappington et al. [78]	2009	C57BL/6J mice, DBA/2J mice and *Rattus norvegicus*	Antagonism of TRPV1 mediates neuroprotection	Low
Leonelli et al. [82]	2010	*Rattus norvegicus*	TRPV1 is involved in the excitotoxic process responsible for RGC death.	Low
Leonelli et al. [81]	2013	*Rattus norvegicus*	TRPV1 agonism promotes NOS expression in retinal tissues in vivo.	Low
Ward et al. [79]	2014	C57BL/6J and *Trpv1*$^{-/-}$ mice	The absence of TRPV1 expression impacts both the anterograde axon transport and the RGCs viability, in the presence of high IOP	Low
Sakamoto et al. [83]	2014	*Rattus norvegicus*	TRPV1 agonism is responsible for RGCs neuroprotection in vivo	Low
Weitlauf et al. [76]	2013	C57BL/6J mice	TRPV1 agonism increase RGCs firing rate in response to elevated IOP	Moderate
Weitlauf et al. [75]	2014	C57BL/6J and DBA/2J mice	TRPV1 increase RGCs excitation in response to elevated IOP	Low
Sappington et al.[77]	2015	Mice, rats and macaque monkeys	TRPV1 might promote RGCs death in response to IOP elevation	Moderate
McGrady et al. [80]	2019	C57BL/6J mice	The absence of TRPV1 expression impacts both RGCs dendritic complexity and total length, in the presence of high IOP.	Moderate

List of abbreviations. RoB: risk of bias; TRPV1: transient receptor potential vanilloid 1; RGC: retinal ganglion cell; IOP: intraocular pressure; NOS: Nitroxide synthase.

aforementioned receptors has been widely explored and has been shown, in certain circumstances, to be comparable or even superior to that of in-the-market hypotensive medications. [44,90] However, while the IOP-lowering properties of the ECS are currently being ascertained, the exact underlying mechanisms remain elusive. For example, CB_1R may exert a dual effect on IOP. In fact, direct agonists of CB_1R are thought to harness aqueous humour production, promoting reduced inflow [91]. However, modulation by inverse agonists appears to be mainly responsible for the weakened resistance to trabecular outflow, possibly via the activation of beta-adrenergic receptors in the TM [38]. Similarly, CB_2R agonism has been shown to be responsible for intracellular and extracellular rearrangements in TM cells (i.e. via a p42/p44-MAP kinase-dependent mechanism), thus inducing enhanced outflow in the porcine perfused anterior segment model [56,57].

Furthermore, a growing body of evidence supports the role of other non-cannabinoid receptors (GPR119 and GPR18) in the IOP-lowering properties of cannabinoid ligands [92,93]. In this regard, even a strict cross-talk between the ECS and the prostanoid pathway should be considered, both exert IOP-lowering properties and depend on the arachidonic acid metabolic cascade [8,31,94]. As a consequence, aqueous outflow via the uveoscleral pathway might be favoured [31,95].

However, when translated into clinical practice, cannabinoids have generally failed to reach significant results in terms of ocular hypotensive effect [96]. Specifically, different compounds (i.e. THC, dronabinol, cannabidiol, PEA, and WIN55212–2) and administration routes (i.e. topical, inhalation, oral, intravenous) have been investigated in humans (for a comprehensive review, see Passani et al. [97]). However, it should be considered that inhaled and intravenous administration of cannabinoids has been linked to systemic adverse events, including postural hypotension, tachycardia, palpitations, and mental status alterations [98]. On the other hand, the topical application of THC did not demonstrate ocular hypotensive efficacy, due to highly lipophilic nature and low solubility of cannabinoids causing a limited corneal permeability and low levels of intraocular penetration [99,100]. In addition, the safety profile of topically applied cannabinoid compounds is compromised by the occurrence of ocular surface irritation and eyelid swelling [97,98]. The short duration of action, low bioavailability, tachyphylaxis, and drug tolerance are other reasons for concern [101,102]. In this context, the adoption of different strategies to improve bioavailability and ameliorate the pharmacokinetics of these highly lipophilic compounds has shown promise [44,45]. However, it should be noted that, as per the aforementioned modes of action, cannabinoids might be more successfully applied as adjuvants to other ocular hypotensive drugs to improve their efficacy. This could be particularly true in

Table 7
List of included articles discouraging the agonism of CB1R, CB2R and TRPV1 as a possible therapeutic strategy for the management of glaucoma. According to our systematic research, 5/33 (15.2%) articles only oppose the use of cannabinoid in glaucoma.

Author	Year	Species	Molecule	Route	Results	Mechanism
Laine et al. [28]	2002	Normotensive pigmented Dutch rabbits	2-AG	Eyedrops	2-AG determines an increase in the IOP followed by a non-statistically significant reduction.	The effect on IOP of 2-AG is probably mediated through a CB_1R-independent mechanism, most probably through its prostanoid metabolites.
Laine et al. [64]	2003	Normotensive pigmented Dutch rabbits	JWH-133	Eyedrops	JWH-133 at the doses of 10 μg and 25 μg did not decrease IOP in the treated eyes.	JWH-133 is not able to determine any hypotensive effect, as being a selective CB_2R agonist. As proposed by the authors, modulation of the IOP by the aforementioned receptor is *not* an effective ocular hypotensive strategy.
Sappington et al. [78]	2009	C57BL/6J mice, DBA/2J mice and Rattus norvegicus	Capsaicin (1 μM)	NA	Increasing doses of capsaicin to RGCs resulted in increased cell death as demonstrated by both the cell count and the TUNEL-assay.	As reported by the authors, the agonism on TRPV1 may favour a potent influx of extracellular Ca^{2+} and a subsequent membrane depolarisation. TRPV1-mediated Ca^{2+} influx leads to many intracellular events, including apoptotic cell death.
Leonelli et al. [81]	2013	*Rattus norvegicus*	Capsaicin (100 μM or higher)	NA	Incubation with capsaicin induced cell death in GCL and INL.	According to the proposed results, the mechanisms involved in TRPV1-mediated cell death include protein nitration and glutamate excitotoxicity.
Leonelli et al. [82]	2010	*Rattus norvegicus*	Capsaicin (100 μM)	NA	After optic nerve transection, treatment with capsaicin determines a thinning of inner retinal layers.	According to the proposed results, the mechanisms involved in TRPV1-mediated cell death include protein nitration and glutamate excitotoxicity.

List of abbreviations. 2-AG: 2-arachidonoylglycerol; IOP: Intraocular Pressure; CB_1R: cannabinoid receptor 1; CB_2R: cannabinoid receptor 2; NA: not applicable; RGCs: retinal ganglion cells; TRPV1: transient receptor potential vanilloid 1.

patients resistant to conventional therapies and in the context of allosteric modulators of cannabinoid receptors, because of the more convenient pharmacokinetic and pharmacodynamic properties [40,103–105].

If the role of the ECS as a regulator of the IOP profile has been extensively explored, only in recent years preclinical research has shed some light on the neuroprotective effects deriving from CB_1R, CB_2R, and TRPV1 modulation in the context of animal glaucoma models [12]. Several mechanisms have been proposed in this regard (Table 7). Among others, the inhibition of the AMPA/glutamatergic excitoxicity-dependent pro-apoptotic cascade and the modulation of the local inflammatory state have been the most explored [52,53,106]. In this context, the ECS-dependent immunomodulatory effects reported in different preclinical studies are particularly intriguing. For example, in an experimental autoimmune uveoretinitis model, the activation of CB_2R was reported to reduce inflammatory mediators in vitro [107,108]. Similarly, Krishnan et al. observed that 2-AG reduced proinflammatory cytokines and increased anti-inflammatory cytokines in Müller glial cultures in a CB_1R- and CB_2R-dependent fashion [109]. These findings are significant because several pro-inflammatory cytokines, such as tumour necrosis factor-α and IL-6, as well as microglial activation, have been reported to play a significant role in glaucomatous RGC death [106,110–112].

However, different other pathways might explain the reported data.

First, it must be considered that cannabinoid receptors are functionally expressed on the membrane of mouse neuronal mitochondria, where they directly control cellular respiration and energy production [113,114]. Mitochondrial dysfunction is one of the key drivers in glaucomatous neurodegeneration, and modulation of ECS-dependent mitochondrial activity might represent a valuable tool promoting neuroprotection and enhancing neuronal function (i.e. neuroenhancement) [115]. In addition, as recently demonstrated, the lack of expression of CB_1R significantly reduces mitophagy activity (i.e. a physiological mechanism responsible for the degradation and recycling of cytoplasmic components through the autophagosomal–lysosomal pathway) in hippocampal neurones, thus altering mitochondrial morphology [116]. Notably, impaired mitophagy has been demonstrated in several neurodegenerative diseases, including glaucoma [117].

In summary, ECS modulation appears to be a promising new target for the treatment of glaucoma as it can directly target the two main drivers of the disease (i.e. IOP and neurodegeneration). However, translational studies conducted *in humans* have not confirmed the constantly growing body of preclinical evidence that supports its application in clinical practice [96,97]. In addition, several issues remain unresolved.

First, the ECS is a highly dynamic and finely tuned regulatory system whose physiology is currently largely obscure [9]. Hence, further research is needed to elucidate the most suitable experimental model and the most convenient molecular mechanism to target and provide the desired hypotensive/neuroprotective efficacy in the context of glaucoma. In this regard, the absence of reliable outcome measures capable of measuring the neuroprotective efficacy of specific compounds further renders research on this topic increasingly challenging.

Moreover, when applied as ocular hypotensive drugs, the short duration of action, low bioavailability, tachyphylaxis, and drug tolerance are major concerns [101,102]. In this context, the adoption of different strategies to improve bioavailability and ameliorate the pharmacokinetics of these highly lipophilic compounds has shown promise [44,45]. Among them, microemulsions with cyclodextrins [118], nanoparticles, and nanoemulsions [45], as well as soluble prodrugs [119] and novel allosteric modulators [120,121] have been variably evaluated both in vitro and in vivo, leading to encouraging preliminary results [103]. Notably, allosteric modulators are agents able to bind portions of receptors, termed allosteric sites, which are different from endogenous ligands [122,123]. Allosteric ligands may promote activation (PAMs) or inhibition (NAMs) of the receptor-signalling cascade. This approach has been reported to be both safe and effective, as described by several studies on the topic [122,123].

However, it should be noted that, as per the aforementioned modes of action, cannabinoids might be more successfully applied as adjuvants to other already available ocular hypotensive drugs to improve their efficacy [38]. This could be particularly true in patients resistant to conventional therapies and the context of allosteric modulators of cannabinoid receptors because of their more convenient pharmacokinetic and pharmacodynamic properties.

However, the psychotropic and non-psychotropic (i.e. bradycardia) systemic effects of several cannabinoid drugs might be considered, particularly those targeting CB_1R [9]. Hence, a pharmacological approach targeting the ECS should not interfere with other organic metabolic pathways.

In addition, it should be noted that the recent development of diverse full, partial, and inverse agonists/antagonists of the endocannabinoid receptors and regulators of both endocannabinoid enzymes and transporter proteins function has shed some light on the eventual different modulatory strategies of the ECS [7]. These could eventually result in pharmacological approaches with only minor systemic and psychotropic side effects.

As per the paucity and conflicting nature of the available data, no specific conclusion can be drawn regarding the expression of CB_1R, CB_2R, and TRPV1, as well as their hypotensive and neuroprotective efficacy in preclinical animal models of glaucoma. Further studies are needed to provide novel insights into this complex through the fascinating aspect of modern ophthalmology.

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CRediT authorship contribution statement

Gabriele Gallo Afflitto: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, **Francesco Aiello:** Data curation, Writing – original draft, **Damiana Scuteri:** Investigation, Writing – review & editing, **Giacinto Bagetta:** Supervision, Writing – review & editing, **Carlo Nucci:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.112981.

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