Review

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Seeing over the horizon – targeting the endocannabinoid system for the treatment of ocular disease

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Abstract: The observation that marijuana reduces intraocular pressure was made by Hepler and Frank in the 1970s. Since then, there has been a significant body of work investigating cannabinoids for their potential use as therapeutics. To date, no endocannabinoid system (ECS)-modulating drug has been approved for clinical use in the eye; however, recent advances in our understanding of the ECS, as well as new pharmacological tools, has renewed interest in the development of ocular ECS-based therapeutics. This review summarizes the current state-of-affairs for the use of ECS-modulating drugs for the treatment of glaucoma and ocular inflammatory and ischemic disease.

Keywords: age-related macular degeneration; cannabinoid receptor 1; cannabinoid receptor 2; cannabinoids; diabetic retinopathy; endocannabinoids; glaucoma; inflammation; ocular; retina; uveitis.

Introduction

Cannabinoids can produce a variety of ocular effects, and prominent among these is a reduction in intraocular pressure (IOP). This latter effect has been the subject of extensive research, primarily with the purpose of developing effective therapeutics for glaucoma, a degenerating eye disease for which ocular hypertension (OH) is a prominent feature [1–5]. Research has now identified that the ocular actions of cannabinoids are mediated by an endogenous endocannabinoid system (ECS) (reviewed in Ref. [3]). Furthermore, in addition to glaucoma, alterations of the ECS have been reported in ocular inflammatory pathologies, including diabetic retinopathy (DR) and age-related macular degeneration (AMD) [6]. This suggests that ECS manipulation may be a promising target for treatment; however, no ECS-modulating drug has yet been approved for clinical use in the eye. Recent advances in our understanding of the ocular ECS, as well as new pharmacological tools, may rectify this situation. This review will discuss recent research that highlights potential new ECS targets for the treatment of ocular disease.

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The ocular endocannabinoid system

The ocular ECS includes enzymes responsible for the production and degradation of endocannabinoids, as well as various receptors that endocannabinoid ligands target, including cannabinoid receptor (CB) 1 and CB2 [3, 7–34]. Additionally, endocannabinoids bind to a variety of "non-classical" cannabinoid receptors, including G-protein-coupled receptor (GPR) 18, GPR55, transient receptor potential vanilloid 1 (TRPV1), and peroxisome proliferator-activated receptors (PPARs) α, β/δ, and γ (reviewed in Ref. [35]). The reported presence of components of the ECS varies by ocular tissue type (see Tables 1–3). For example, the retina, the neuronal tissue responsible for the generation of vision, highly expresses various endocannabinoid-binding receptors and enzymes, whereas other tissues like the lens, the crystalline structure responsible for the focusing of light on the retina, is apparently devoid of ECS components [3, 7, 24].

2-Arachidonoylglycerol (2-AG) and anandamide (*N*-arachidonoyl ethanolamine; AEA) are two of the most

Table 1: Presence and localization of endocannabinoids in the mammalian eye.

	Retina	Iris	TM	SC	CB	Choroid	Species	References
AEA	+	+	+		+	+	Cow, human, pig, rat	Matsuda et al. [7]; Bisogno et al. [9]; Stamer et al. [15]; Chen et al. [17], but see Straiker et al. [10]
2-AG	+	+			+	+	Cow, human, rat	Bisogno et al. [9]; Straiker et al. [10]; Chen et al. [17]
PEA	+	+			+	+	Cow, human, rat	Bisogno et al. [9]; Straiker et al. [10]; Chen et al. [17]

+, Endocannabinoid is present in tissue; TM, trabecular meshwork; SC, Schlemm's canal; CB, ciliary body.

Table 2: Presence and localization of endocannabinoid synthesizing and degrading enzymes in the mammalian eye.

	Retina	Iris	TM	SC	CB	Choroid	Species	References
DGLα/β	+[a]						Mouse, rat	Hu et al. [24]; Zabouri et al. [26]
NAPE-PLD	+						Mouse, rat	Zabouri et al. [26]; Cécyre et al. [33]
FAAH	+		+				Cow, monkey, mouse, rat	[b]Bisogno et al. [9]; Yazulla [3]; Yazulla et al. [12]; Njie et al. [22]; Hu et al. [24]; Bouskila et al. [27]
MGL	+		+				Mouse, rat	Njie et al. [21]; Yazulla [3]; Hu et al. [24]
ABHD6	+						Rat	Hu et al. [24]

+, Protein expression (immunohistochemistry, Western blotting); TM, trabecular meshwork; SC, Schlemm's canal; CB, ciliary body. [a]DGLβ was associated only with blood vessels in the retina [24]. [b]Pharmacological evidence only.

Table 3: Presence and localization of classical and non-classical cannabinoid-binding receptors in the mammalian eye.

	Retina	Iris	TM	SC	CB	Choroid	Species	References
CB1	+, ‡	+, ‡	+, ‡	+	+, ‡	‡	Guinea pig, human, mouse, monkey, pig, rat	Porcella et al. [8, 14]; Straiker et al. [10, 11]; Yazulla et al. [12]; Stamer et al. [15]
CB2	+, ‡		+				Monkey, pig, rat	Lu et al. [13]; Zhong et al. [18]; [a]He et al. [19]; Lopez et al. [25]; Cécyre et al. [33], but see Porcella et al. [8], and Bouskila et al. [30]
GPR18	+, ‡	+	+		+	+	Mouse, rat	Caldwell et al. [32]; MacIntyre et al. [34]
GPR55	+[b]		+				Monkey, pig	Kumar et al. [28]; Bouskila et al. [31]
TRPV1	+, ‡						Cat, monkey, rat	Yazulla and Studholme [16]; Nucci et al. [20]; Sappington et al. [23]
PPARα	‡						Cow, pig	Kumar et al. [28]; [c]Romano and Lograno [29]

+, Protein expression (immunohistochemistry, Western blotting); ‡, mRNA expression (RT-PCR). TM, Trabecular meshwork; SC, Schlemm's canal; CB, ciliary body. [a]Pharmacological evidence only. [b]Staining exclusive to rods. [c]Pharmacological data from ophthalmic artery only.

well-studied endocannabinoids. Both 2-AG and AEA are found throughout ocular tissues, and like elsewhere in the central nervous system (CNS), 2-AG is present in higher concentrations than AEA [6, 36, 37]. Additionally, *N*-palmitylethanolamide (PEA), an analogue of AEA, has also been found in ocular tissues (see Table 1) [6, 17]. Endocannabinoid levels are maintained by the balance between on-demand synthesis and degradation. Endocannabinoid biosynthetic and degradative enzymes including diacylglycerol lipase (DGL) α/β, *N*-arachidonoyl phosphatidylethanolamine phospholipase D (NAPE-PLD), fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGL), and α/β-hydrolase domain 6 (ABHD6) have all been localized to the mammalian eye ([12, 24, 26]; see Table 2 for a summary of expression).

Expression of CB1 is ubiquitous throughout the eye [8, 10–12, 14, 15]; however, both the presence and location of CB2 expression in a non-pathological state is controversial. Some pharmacological evidence has suggested that CB2 is present on the trabecular meshwork and therefore may be involved in aqueous humor dynamics [18, 19]. Furthermore, while several groups have reported finding CB2 mRNA and positive immunoreactivity throughout the retina [13, 25, 33], others have found CB2 immunoreactivity restricted to Müller cells [30].

Interpretation of published CB2 studies are difficult in that at least some drugs that act at CB2 also display activity at other receptors. For example, the CB2 "selective" drug (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone (JWH 015) was recently shown to bind to CB1 at relatively

low concentrations [38]. Additionally, another recent study has raised the question of whether or not the CB2 antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR144528) may also exhibit non-CB1/CB2-mediated actions [28]. Furthermore, immunohistochemical studies reporting CB2 in the eye have also documented variable immunoreactivity depending on antibody lot [33]. Therefore, further documentation of receptor localization and function are still required in order to arrive at firm conclusions regarding the role(s) of CB2 in the retina and ocular tissues.

More recently, the cannabinoid-binding receptors GPR55, GPR18, TRPV1, and PPARα have also been localized within ocular tissues [16, 20, 23, 28–30, 32, 34]. A brief summary of the localization of these receptors in ocular tissues is located in Table 3.

The function of the ocular ECS is not fully understood; however, there are significant data to suggest that it may be important in visual processing, IOP control, as well as modulating inflammation [3, 33, 39]. Additionally, fluctuations in endocannabinoid levels have been measured during several disease states and may actually contribute to disease pathology or its resolution (reviewed in Ref. [40]).

Cannabinoids, intraocular pressure, and glaucoma

Hepler and Frank [41] first described the IOP-lowering effects of smoked marijuana in 1971. Since then, ECS-modulating drugs have been extensively investigated as potential therapeutics for the treatment of OH [1, 2, 4, 39], a risk factor for glaucoma [42]. Glaucoma, the second leading cause of blindness worldwide, is a progressive neurodegenerative disorder characterized by retinal ganglion cell (RGC) loss [5]. However, the mechanisms leading to RGC death in glaucoma are not yet fully understood; IOP is the only modifiable risk factor and therefore is currently the only target for therapy [43–45]. Success of cannabinoids for the treatment of OH in clinical trials has been limited, primarily due to their variable efficacy, most likely a result of their short duration of action in reducing of IOP, potential receptor desensitization, and behavioral side effects [4]. The following sections will summarize the current state-of-affairs for the use of ECS-modulating drugs for control of IOP as well as recent evidence supporting cannabinoid-mediated (and IOP-independent) RGC neuroprotection in glaucoma.

Intraocular pressure

Components of the ECS are highly expressed on tissues responsible for IOP regulation [2, 4, 39]. IOP is the product of the difference between aqueous humor production and outflow. Aqueous humor is a clear liquid that serves as a circulatory system within the eye, nourishing and removing wastes from avascular areas of the eye, and helps maintain eye shape, which is important for proper optics. Aqueous humor is produced by the bilayered ciliary epithelium, and once formed, flows into the posterior chamber before circulating to the anterior chamber (reviewed in Ref. [46]). Outflow is complex and occurs through either the conventional pathway, which flows from the anterior chamber through the trabecular meshwork and into Schlemm's canal, or through the uveoscleral pathway, which involves the flow of aqueous humor from the irideocorneal angle through to the ciliary body before draining into the supraciliary and suprachoroidal spaces [47]. The amount of flow through either the uveoscleral pathway or the classical pathway is dynamic and varies significantly between species. As such, the development of new IOP-modulating drugs may be confounded by species differences; an effective ocular hypotensive in one species, such as rabbits, may not necessarily translate into significant changes in IOP in humans [47].

The effects of the phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) and the synthetic agonist (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN 55,212-2; WIN) on IOP have been well studied [48–52]. These cannabinoids appear to lower IOP by a CB1-mediated mechanism that is β-adrenergic receptor dependent [53, 54], similar to what was found elsewhere [55]. So far, there has been a limited number of human studies with variable results using Δ^9-THC and WIN in clinical trials [52, 56]; however, new therapeutic strategies and targets, combined with localized drug delivery, may provide better methods to increase the efficacy and duration of IOP-lowering actions, while reducing systemic and CNS side effects.

Neuroprotection

There is currently no approved glaucoma therapeutic that directly targets RGC loss. Such a drug would be beneficial, as many patients with glaucoma continue to have progressive visual field loss despite IOP control [45]. Furthermore, patients where IOP never becomes elevated but have

glaucoma (so-called normal tension glaucoma) may also benefit from a neuroprotective-targeted therapy [45].

Triggers that ultimately lead to RGC death in glaucoma are likely multifactorial, although consistent evidence suggests that this death ultimately occurs via caspase-dependent apoptosis [57]. Once caspases are activated, the retinal damage is irreversible [58], and therefore, potential neuroprotective targets must be upstream of caspase activation. Several hypotheses have been proposed as mechanisms leading to RGC loss. These include excitotoxicity, loss of neurotrophic support at the optic nerve, and oxidative stress, and it is likely that more than one of these factors may be contributing (as reviewed in Ref. [57]). Given the complexities of RGC death in glaucoma, the fact that ECS-modulating drugs can target multiple signaling pathways could be advantageous [59, 60]. The neuroprotective mechanisms of ECS-modulating drugs include decreasing glutamatergic signaling via presynaptic modulation of voltage-gated Ca^{2+} channels and K^{+} channels with a resultant reduction in neurotransmitter release [61–63], activation of pro-survival pathways, such as by activation of protein kinase B (Akt) and extracellular signal-regulated kinases (ERK) 1/2 [64–67], and reduction of immune cell (e.g. resident microglia) activation and migration, thereby decreasing nitric oxide production and release of pro-inflammatory cytokines [68–72].

Administration of cannabinoid drugs has produced RGC neuroprotection in a number of different models [73–76]. Notably, Pinar-Sueiro et al. [75] showed significant neuroprotection with topical administration of WIN in a model of transient high-IOP ischemia-reperfusion injury. The WIN-induced increase in RGC survival was blocked with co-administration of the CB1 antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM 251), suggesting a CB1-mediated mechanism. However, as this model involves changes in aqueous humor dynamics and WIN-induced reductions in IOP were not measured, it is possible that this neuroprotective effect was in part IOP-mediated. Meanwhile, Slusar et al. [37] showed that systemic administration of cyclohexylcarbamic acid 3′-(aminocarbonyl)-[1,1′-biphenyl]-3-yl ester (URB 597), an inhibitor of the AEA degrading enzyme FAAH, was neuroprotective in a rat axotomy model, an IOP-independent model of RGC loss. This effect was inhibited by the CB1 antagonist AM 251, but not the CB2 antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone (AM 630), suggesting that this effect was also CB1-dependent [37]. Investigation of the neuroprotective effect of ECS-modulating drugs is still relatively new. With a better understanding of the mechanisms leading to RGC death, further development of ECS-modulating drugs may lead to more effective therapeutics for the treatment of glaucoma.

New horizons in glaucoma?

CB1 agonists that reduce IOP, such as WIN and Δ^9-THC, have been well studied as potential glaucoma therapeutics in both preclinical and clinical studies [48–52, 77]. However, to date, no cannabinoids drug has been approved for the treatment of glaucoma. This lack of success of cannabinoid therapeutics in glaucoma is largely attributed the short duration of action and possible behavioral and systemic side effects of cannabinoids, and the existence of alternative effective ocular hypotensive medications [43, 78, 79]. More recently, several new pharmacological strategies have been developed, which may reduce the limitations of CB1 agonists. Some of these strategies include the use of biased agonists or allosteric modulators and the use of degradative enzyme inhibitors [80–82]. In particular, modulating CB1 through allosteric modulation and/or inhibition of endocannabinoid-degrading enzymes (like FAAH and MAGL) may be able to increase duration of action, reduce desensitization, and limit psychotropic effects [80, 82–85].

The effects of administered endocannabinoids such as 2-AG and AEA are extremely short-lived and therefore not ideal as a therapeutic strategy [22, 53]. One method to increase the duration of effect would be to increase the amount of endocannabinoid available at the receptor through the use of inhibitors of endocannabinoid metabolism [82–84]. For example, Njie et al. [22] demonstrated that AEA administered in a porcine ocular perfusion model produced an increase in outflow facility, but this effect only persisted for 30 min. However, when AEA was co-administered with URB 597, a FAAH inhibitor, this effect was prolonged to 5 h [22]. Similarly, in another study using the same model, administration of the non-selective MGL/FAAH/ABHD6 inhibitor 5-[(1,1′-biphenyl]-4-yl)methyl]-*N*,*N*-dimethyl-1*H*-tetrazole-1-carboxamide (LY 2183240) increased outflow facility with a comparable duration of action to that seen with AEA+URB 597 [21]. Use of URB 597 was also found to be neuroprotective in an acute model of transient high-IOP retinal ischemia-reperfusion injury [20] and a model of IOP-independent RGC loss [37]. The neuroprotective effects of URB 597 were independent of concomitant exogenous AEA dosing [20, 37].

Although to the best of our knowledge there are no published studies on the use of selective inhibition of 2-AG metabolizing enzymes (such as MGL and ABHD6)

in a model of glaucoma, other studies have examined neuroprotective effects of inhibiting 2-AG degradation. These include a model of traumatic brain injury, where a significant reduction in lesion size was found with administration of the ABHD6 inhibitor *N*-methyl-*N*-[[3-(4-pyridinyl)phenyl]methyl]-4′-(aminocarbonyl) [1,1′-biphenyl]-4-yl carbamic acid ester (WWL 70) [86]. Additionally, in an experimental model of Parkinson disease, significant neuroprotection occurred after administration of the MGL inhibitor 4-[*bis*(1,3-benzodioxol-5-yl) hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester (JZL 184) [87, 88]. Taken together, these results suggest that use of enzyme-inhibiting drugs may be more appropriate for chronic treatment of neurodegeneration than administration of endocannabinoids alone.

An allosteric site on CB1 was recently characterized [89], and since then, a few allosteric-modulating drugs have been investigated [80, 85, 90–93]. Allosteric modulators bind to a site other than the natural, or orthosteric, binding site. Compounds binding to the allosteric site can affect the affinity and/or efficacy of the orthosteric ligand to the receptor (e.g. endocannabinoids to CB1) [94]. In doing so, allosteric modulators decrease the potential of CB1-mediated psychotropic effects by producing a more favorable therapeutic index (decreased dose) for the use of CB1 orthosteric ligands with reduced receptor desensitization (improved long-term efficacy) [80]. Furthermore, as upregulation of cannabinoid receptors has been reported in various disease states, including the eye [20], use of positive allosteric modulators (PAMs) may prove effective for increasing the localized actions of endocannabinoids at specific tissue sites [80]. So far, relatively few studies have been published using CB1 PAMs, and none yet so far in the eye. Pamplona et al. [85] studied the use of the endogenous CB1 PAM lipoxin A_4 in a model of β-amyloid-induced memory impairment. Mice receiving lipoxin A_4 performed significantly better in a spatial memory task compared with controls, possibly suggesting some neuroprotective effect. Although work in this area is still in the very early stages, the potential for these types of drugs in chronic disease is large and therefore is an important area of study.

Another new ECS-modulating drug strategy has been the use of ligand-directed, or biased, signaling [81, 93]. CB1 couples to three major Gα proteins (G_i, G_o, and G_s) and can therefore elicit varied signaling pathways (reviewed in Ref. [95]). Studies analyzing downstream signaling of CB1 ligands have found that not all CB1 agonists activate the G proteins in the same manner [81, 96, 97]. Use of ligands that are biased for a particular pathway may be able to elicit favorable results, while minimizing side effects [98]. Typically, CB1 activates G_i-mediated pathways; however, activation of CB1 by WIN has also been reported to activate G_q- and G_s-mediated pathways (reviewed in Refs. [81, 95]). Additionally, CB1 has been shown to dimerize to a number of different receptors including A_{2A} adenosine receptor, β_2 adrenergic receptor, D_2 dopamine receptor, orexin 1 receptor, and μ, κ, and δ opioid receptors, which may result distinct G-protein- or non-G-protein-biased signaling depending on the receptor interactions (reviewed in Refs. [81, 95]). Future work will determine which of these signaling pathways will provide the most therapeutic benefit and allow further development of biased ligands for specific ocular diseases.

Apart from CB1, studies of other ECS targets have been examined for the treatment of glaucoma. These include the non-CB1/CB2 receptors TRPV1, GPR18, and PPARα, all of which can bind cannabinoids [35]. TRPV1 is a cation-selective channel, expressed on both neuronal and microglial cells in the retina [16, 20, 23]. TRPV1 is activated by the endocannabinoid AEA, as well as vanilloids, and is suggested to be important in cellular excitability (reviewed in [82, 99]). Investigation of the role of TRPV1 in pressure-induced RGC death has led to some opposing results, calling into question the exact role of TRPV1 in the retina [23, 99–103]. Depending on the study, researchers have concluded that TRPV1 activation can either be neuroprotective [103] or is a major contributor to pressure-induced damage in the eye [23, 100]. The expression of TRPV1 in models of ischemia/reperfusion and OH has been varied. However, recent work found that RGC damage appears to be exacerbated in $TRPV1^{-/-}$ mice [103], suggesting that TRPV1 in the retina is neuroprotective. Mechanisms of TRPV1 activation to promote RGC survival are complex, but may involve promotion of RGC excitability during retinal stress, as well as release of neuroprotective cytokines, such as interleukin (IL) 6, from glial cells (as reviewed in Refs. [99, 102]). Work investigating the role of TRPV1 in models of glaucoma is ongoing; however, its manipulation, either on its own or in conjunction with other cannabinoid receptors, holds therapeutic potential.

GPR18 is a recently deorphanized G-protein-coupled receptor for which *N*-archidonoylglycine (a metabolite of AEA) and abnormal cannabidiol (Abn-CBD) are ligands [104, 105]. GPR18 is expressed in the retina, ciliary epithelium, corneal epithelium, and trabecular meshwork [32, 34]. Application of GPR18 agonists reduced IOP independently of CB1, CB2, and GPR55 [32, 105]. Additionally, this IOP-lowering effect was independent of β-arrestin 1 and 2 [32], unlike the actions of CB1 [53]. This suggests that drugs modulating GPR18 may be a good target for IOP modulation by a distinct mechanism than CB1 and

therefore devoid of psychotropic side effects. In the retina, GPR18 co-localizes with retinal microvasculature and application of its agonists causes vasorelaxation [34]; however, the effects of GPR18 in retinal function have yet to be studied.

PEA is an analogue of AEA; however, this endocannabinoid does not bind to either CB1 or CB2, but competes with AEA for degradation by FAAH. Although increases in endogenous PEA levels could potentially result in increases in AEA and AEA-mediated activation of CB1, when administered topically PEA does not lower IOP [106]. However, two small clinical trials have investigated the effect of oral PEA in glaucoma. One clinical trial involved oral administration of PEA to patients with primary open angle glaucoma and found a significant reduction in IOP compared with baseline measurements [107]. Another small clinical trial found similar results in patients with normal tension glaucoma: IOP was reduced and visual fields parameters improved when measured at a 6-month follow-up [108]. Some effects of PEA have been attributed to actions at non-CB1/CB2 receptors. A study by Kumar et al. [28] found that PEA increased ocular outflow facility in a perfused porcine model, an effect partially attributed to PPARα activation. Interestingly, the authors of the article [28] also concluded that this PEA-induced increase in outflow also involves GRP55, as shRNA knockdown of GPR55 partially attenuated the effect [28]. In contrast, an IOP-lowering effect remained intact in GPR55 knockout mice when administered the non-selective GPR55 agonist Abn-CBD, suggesting that this receptor is not involved in IOP regulation [32]. Additionally, activation of PPARα by PEA (and AEA) caused vasorelaxation of bovine ophthalmic artery and was blocked by inhibitors of nitric oxide synthase and large conductance Ca^{2+}-activated K^+ channels [29]. Further, PEA administration after traumatic CNS injury is neuroprotective, potentially by decreasing edema and modulation of inflammation (as reviewed in Ref. [109]). Taken together, this evidence suggests that PEA may be effective at lowering IOP by a mechanism independent of CB1 and CB2 and that given the neuroprotective actions of PEA reported, future studies to analyze the effect of PEA on RGCs are warranted.

The use of ECS-modulating drugs for the treatment glaucoma remains an as yet unrealized area. As demonstrated by the recent research, there is significant new evidence that warrants further study into novel ECS-based therapeutics, targeting both IOP and RGC loss. Given that there is currently no treatment that directly targets the RGC loss in glaucoma, the potential of ECS-based therapeutics for ocular neurodegenerative disease merits further exploration in both appropriate experimental models and in clinical studies.

The endocannabinoid system and ocular inflammation

Cannabinoid researchers have been actively investigating the role of the ECS in the immune response since 1993 [110]; however, until very recently, its role in the ocular immune response has not been examined. The ECS is a potential therapeutic target for immunomodulation with a growing body of evidence indicating that both CB1 and CB2 signaling may contribute (reviewed in Refs. [60, 111, 112]). Activation of CB2, specifically, is anti-inflammatory in a number of tissues and organs, including the eye [113–115]. CB2 was cloned from a human promyelocytic leukaemia cell line HL60 and rat macrophages and was referred to as the peripheral cannabinoid receptor [110, 116]. CB2 has since been localized to all examined subtypes of leukocytes [117–121], in addition to other non-immune cells, including endothelial cells [122], osteoclasts [55], with reports in neurons and peripheral nerve terminals [60]. Within the subsets of immune cells, CB2 mRNA levels vary being highest in B-cells>natural killer cells>monocytes>polymorphonuclear cells>T8 cells>T4 cells [117]. CB2 is also expressed by antigen-presenting cells (APCs), including macrophages, dendritic cells, and microglia [123, 124]. CB2 immunomodulation has been extensively reviewed by others [120, 125, 126]. Briefly, it has been shown that CB2 agonists can decrease release of inflammatory mediators including cytokines, chemokines, and adhesion molecules, inhibit chemotaxis of immune cells, and modulate proliferation and antigen presentation [118, 119, 121, 124, 127]. Furthermore, during inflammation, CB2 expression is increased in vivo and in vitro, and this may result in alternations in CB2 signaling [128, 129].

The ocular immune system

The eye is a unique organ of the body, encompassing both the peripheral nervous system (PNS) and CNS immune system. Immune cells associated with the peripheral region of the eye, including the cornea, ciliary body, iris, choroid, include populations of cells such as macrophages and dendritic cells [130–132]. The retina is an extension of the CNS and thus includes resident immune cells such as microglia [133]. The complexity of the ocular immune system is increased by the immune privilege status of the

eye, which is achieved by a physical blood-ocular barrier (formed by the blood-aqueous barrier and the blood-retinal barrier [BRB]), absence of lymphatic pathways, immunomodulatory factors released in the aqueous humor, immunoregulation via cell-to-cell contact mechanisms with corneal endothelium and iris-pigmented epithelium, and APC development of antigen tolerance [133–137]. Together, these adaptations help to maintain the ocular microenvironment and proper ocular function.

Following trauma or inflammation in the eye, the blood-ocular barriers are compromised, and resident APCs, including macrophages, dendritic cells, and microglia, activate and recruit other immune cells of innate and adaptive immune systems. This recruitment is facilitated by cytokines and chemokines. In the case of ocular innate immune response, neutrophils, basophils, eosinophils migrate from systemic circulation to the site of inflammation [137]. The adaptive immune response is based on APCs processing and displaying antigens, which are detected and recognized by T-cells to elicit their activation [138]. Both systems are intricately linked together and constitute the cells that make up the ocular immune system.

Cannabinoids and uveitis

The immune response within the eye can become exacerbated during inflammatory disease states such as uveitis and override the immune privilege compensatory mechanism, threatening eyesight. Uveitis is a broad term that describes inflammation, arising from either infectious or non-infectious origins, occurring within specific regions of the eye including the uvea, which comprises the iris, ciliary body, and choroid. Additionally, inflammation within the sclera, retina, vitreous, and optic nerve can also be classified as uveitis. The location of the inflammation within different anatomical regions allows several designations for the type of uveitis: anterior, intermediate, and posterior or throughout the eye (pan-uveitis [139]). Current pharmacological treatments for uveitis include classical immunosuppressive drugs, such as corticosteroids, as well as newer biological agents. However, chronic corticosteroid use can lead to numerous adverse effects, including cataracts and glaucoma, and biologicals are also extremely costly [140, 141].

Several recent studies have indicated the potential of the ECS, and in particular CB2, as a therapeutic target to treat uveitis and other ocular inflammatory and fibrotic diseases [113, 114]. The anti-inflammatory effects of selective CB2 agonist, (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (JWH 133), have been examined in experimental uveitis [113]. This study used a pan-uveitis model based on a CD4+ T-cell-mediated response to interphotoreceptor binding protein peptide, with a primary focus on pathological changes in the retina. JWH 133 dose-dependently improved both clinical and histopathological scores with respect to inflammatory cell infiltration and retinal structural changes. This was accompanied by blunted cytokine production, including interferon (IFN) γ, tumor necrosis factor (TNF) α, IL-6, and IL-10, and completely ablated monocyte chemoattractant protein 1 (CCL2) production in JWH 133-treated animals. Xu et al. [113] attributed these anti-inflammatory effects to the direct actions of JWH 133 on lymphocytes.

More recently, Toguri et al. [114] further demonstrated the potential of CB2 agonists as anti-inflammatory agents during uveitis. This study used real-time non-invasive intravital microscopy to measure leukocyte-endothelial adhesion in the iridial microcirculation in a model of endotoxin-induced uveitis by intraocular injection of lipopolysaccharide (LPS). Topical application of the cannabinoid 4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol (HU 308), a CB2 agonist, significantly decreased leukocyte-endothelial adhesion, and this effect was blocked by the CB2 antagonist AM 630. Toguri et al. [114] reported that CB2 activation reduced transcription factors nuclear factor κB and activator protein 1, accompanied by decreased cytokines (including TNF-α, IL-1β, IL-6, IL-10, IFN-γ) and chemokines (CCL5 and CXCL2). These effects were antagonized by AM 630. Notably, in this study, CB2-activating drugs performed better than three clinically used anti-inflammatory drugs to treat uveitis, e.g. dexamethasone, prednisolone, nepafenac.

Relatively few studies to date have specifically examined the actions of endocannabinoids in uveitis. In contrast to other studies that reported the anti-inflammatory actions of cannabinoids in experimental uveitis, Altinsoy et al. [142] reported that introduction of AEA via intraocular injection in a rabbit model of LPS-induced uveitis exacerbated all measured outcomes of inflammation. The increase in inflammation seen with AEA was reduced by CB1 antagonism. In contrast to Altinsoy et al. [142], Toguri et al. [115] recently reported that the cannabinoid WIN, given via the intravenous route, reduces leukocyte recruitment in experimental uveitis generated by systemic LPS injection in rats, and this beneficial effect of WIN was reduced by both CB2 and CB1 antagonism. In support of this latter study, a reduction in retinal damage was also reported following treatment with either WIN or Δ^9-THC

(intraperitoneal route) in a model of experimental autoimmune uveoretinitis in mice [143].

Taken together, the evidence supports an anti-inflammatory action of CB2 in uveitis. However, the anti-inflammatory actions of non-selective cannabinoids may also include CB1 activation on both immune cells and vasculature [115]. These findings strongly support the development of ECS-modulating drugs in the treatment of ocular inflammation.

Cannabinoids, diabetic retinopathy, and age-related macular degeneration

DR is a retinal disease associated with chronic inflammation and neovascularization (reviewed in Ref. [144]). Breakdown of the BRB and retinal cell death are clinical features of DR, and are thought to occur as a result of hyperglycemia-induced oxidative stress leading to increased release of proinflammatory cytokines (reviewed in Ref. [145]). A number of studies have examined the involvement of the ECS and cannabinoids in experimental rodent DR models. The effects of the cannabidiol (CBD) were examined in DR induced in rats by injection of streptozotocin [146]. Chronic CBD treatment was anti-inflammatory and resulted in a significant decrease of BRB breakdown, oxidative stress, and vascular endothelial growth factor expression. This study also reported a reduction in activated p38 mitogen-activated protein kinase (MAPK), a stress-activated protein kinase that is a downstream target of proinflammatory cytokines and oxidative stress and a reduction in retinal cell death [146]. The receptor target for the actions of CBD in the retina was not extensively explored in this study; however, other mechanistic studies have reported pleiotropic actions of CBD including antagonism of CB1, as well as agonist actions at adenosine A_{2A} receptors, 5-hydroxytryptamine 1A receptors, and nuclear PPARγ receptors (reviewed in Ref. [147]). In keeping with a potential CB1 antagonistic role of CBD, a more recent study by El-Remessy et al. [148] reported that genetic deletion or chronic receptor blockade of CB1 in a mouse model of DR prevented retinal cell death. Blocking CB1 receptor signaling in these models was associated with reduced oxidative stress, Müller cell activation, as well as decreased levels of proinflammatory cytokines and adhesion factors (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) and reduced activation of stress signaling pathways p38 and Jun N-terminal kinase MAPKs.

Although endocannabinoid and cannabinoid receptor protein levels were not reported in these rodent DR models, Matias et al. [6] examined endocannabinoid levels in post-mortem eyes from patients with DR. The authors reported elevated AEA levels in the retina, which they attributed to dysfunctional ECS signaling. In the same study, the authors also found elevated AEA levels in retinas of post-mortem eyes of patients with AMD. AMD is a degenerative retinal disease leading to retinal pigmented epithelium (RPE) atrophy, in which oxidative stress is a contributing factor [149]. Exposing primary human RPE cells and an RPE cell line to hydrogen peroxide-induced oxidative stress upregulated expression of CB1 and CB2 receptors and downregulated FAAH expression [149]. This latter finding would imply that decreases in FAAH could contribute to the reported increased levels of AEA in post-mortem AMD eyes.

Although there is a lack of information on the effects of cannabinoids in in vivo preclinical models of AMD, Wei et al. [149] reported expression of CB1 and CB2 receptors as well as FAAH in human RPE cells. Treatment of RPE cells with cannabinoids, including the CB1 and CB2 agonist CP 55,940 and the CB2 agonist JWH 015, protected cells from oxidative stress-induced cell death by reducing ROS and activating of P13K/Akt pro-survival signaling pathways. In a follow-up study, Wei et al. [150] investigated the effects of the selective CB1 receptor antagonist SR 141716 and receptor inhibition by siRNA in primary human RPE cells exposed to hydrogen peroxide-induced oxidative stress. Their findings suggest that attenuating CB1 receptor signaling ameliorates oxidative stress-induced cell death and enhances activation of P13K/Akt [150]. These studies support the hypothesis that cannabinoid signaling plays a role in AMD. However, further studies in retinal ischemic pathologies such as DR and AMD are clearly required to resolve changes in ECS signaling in these pathologies as well as to determine the most appropriate therapeutic approach.

Final thoughts

Through the development of better pharmacological tools and an increased understanding of the ocular ECS, there is significant potential for the development of new ECS-targeted therapies for ocular diseases. With regard to glaucoma, drugs targeting cannabinoid receptors, such as CB1, may be advantageous as adjuncts to existing clinical hypotensive agents due to their dual actions in lowering IOP and providing neuroprotection of RGCs. Furthermore, development of CB1 allosteric modulators and enzyme inhibitors for ocular use would maintain the benefits of CB1 activation, and may result in a more favorable therapeutic index [80, 82–85].

The ECS is important in the modulation of the ocular immune response [113, 114]. Studies of CB1 in inflammation

have variable results; however, in the treatment of ocular inflammation, there is convincing evidence that suggests that CB2 may be a clinically relevant target [114, 142]. So far, most research has been focused on modulating specific components of the ocular inflammatory response. However, given that the eye has both elements of the PNS and CNS immune responses, as well as both innate and adaptive immune systems, additional studies encompassing these complexities are still required before a CB2 agonist or other ECS modulators can reach the clinic [130–133]. Additional studies examining ECS modulation in ocular tissues in which both receptors and endocannabinoids have been identified, e.g. the cornea, may provide evidence for additional analgesic and anti-inflammatory therapeutic indications [6, 11, 151–153].

A significant amount of work in the last few years has granted new insights into the function of the ocular ECS, particularly in ocular disease. However, not all observed effects of ECS-modulating drugs can currently be fully explained, and it appears that in some cases we may be missing parts of the puzzle. Further studies encompassing the complexities of both the ECS and ocular disease pathologies will enable a better understanding of ECS-modulating drug actions and may enable the generation of better targeted and effective therapeutics. Given the current momentum of discoveries in this area, it is quite possible that ECS-modulating drugs could soon be clinically available for the treatment of ocular disease.

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References

1. Green K. Marihuana in ophthalmology – past, present and future. Ann Ophthalmol 1979;11:203–5.
2. Jarvinen T, Pate DW, Laine K. Cannabinoids in the treatment of glaucoma. Pharmacol Ther 2002;95:203–20.
3. Yazulla S. Endocannabinoids in the retina: from marijuana to neuroprotection. Prog Retin Eye Res 2008;27:501–26.
4. Pinar-Sueiro S, Rodriguez-Puertas R, Vecino E. Cannabinoid applications in glaucoma. Arch Soc Esp Oftalmol 2011;86:16–23.
5. King A, Azuara-Blanco A, Tuulonen A. Glaucoma. Br Med J 2013;346:f3518.
6. Matias I, Wang JW, Moriello AS, Nieves A, Woodward DF, Di Marzo V. Changes in endocannabinoid and palmitoylethanolamide levels in eye tissues of patients with diabetic retinopathy and age-related macular degeneration. Prostaglandins Leukot Essent Fatty Acids 2006;75:413–8.
7. Matsuda S, Kanemitsu N, Nakamura A, Mimura Y, Ueda N, Kurahashi Y, et al. Metabolism of anandamide, an endogenous cannabinoid receptor ligand, in porcine ocular tissues. Exp Eye Res 1997;64:707–11.
8. Porcella A, Casellas P, Gessa GL, Pani L. Cannabinoid receptor CB1 mRNA is highly expressed in the rat ciliary body: implications for the antiglaucoma properties of marihuana. Brain Res Mol Brain Res 1998;58:240–5.
9. Bisogno T, Delton-Vandenbroucke I, Milone A, Lagarde M, Di Marzo V. Biosynthesis and inactivation of N-arachidonoylethanolamine (anandamide) and N-docosahexaenoylethanolamine in bovine retina. Arch Biochem Biophys 1999;370:300–7.
10. Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ, Maguire G. Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc Natl Acad Sci USA 1999;96:14565–70.
11. Straiker A, Maguire G, Mackie K, Lindsey J. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Invest Ophthalmol Vis Sci 1999;40:2442–8.
12. Yazulla S, Studholme KM, McIntosh HH, Deutsch DG. Immunocytochemical localization of cannabinoid CB1 receptor and fatty acid amide hydrolase in rat retina. J Comp Neurol 1999;415:80–90.
13. Lu Q, Straiker A, Lu Q, Maguire G. Expression of CB2 cannabinoid receptor mRNA in adult rat retina. Vis Neurosci 2000;17:91–5.
14. Porcella A, Maxia C, Gessa GL, Pani L. The human eye expresses high levels of CB1 cannabinoid receptor mRNA and protein. Eur J Neurosci 2000;12:1123–7.
15. Stamer WD, Golightly SF, Hosohata Y, Ryan EP, Porter AC, Varga E, et al. Cannabinoid CB1 receptor expression, activation and detection of endogenous ligand in trabecular meshwork and ciliary process tissues. Eur J Pharmacol 2001;431:277–86.
16. Yazulla S, Studholme KM. Vanilloid receptor like 1 (VRL1) immunoreactivity in mammalian retina: colocalization with somatostatin and purinergic P2X1 receptors. J Comp Neurol 2004;474:407–18.
17. Chen J, Matias I, Dinh T, Lu T, Venezia S, Nieves A, et al. Finding of endocannabinoids in human eye tissues: implications for glaucoma. Biochem Biophys Res Commun 2005;330:1062–7.
18. Zhong L, Geng L, Njie Y, Feng W, Song ZH. CB2 cannabinoid receptors in trabecular meshwork cells mediate JWH015-induced enhancement of aqueous humor outflow facility. Invest Ophthalmol Vis Sci 2005;46:1988–92.
19. He F, Song ZH. Molecular and cellular changes induced by the activation of CB2 cannabinoid receptors in trabecular meshwork cells. Mol Vis 2007;13:1348–56.

20. Nucci C, Gasperi V, Tartaglione R, Cerulli A, Terrinoni A, Bari M, et al. Involvement of the endocannabinoid system in retinal damage after high intraocular pressure-induced ischemia in rats. Invest Ophthalmol Vis Sci 2007;48:2997–3004.
21. Njie YF, He F, Qiao Z, Song ZH. Aqueous humor outflow effects of 2-arachidonylglycerol. Exp Eye Res 2008;87:106–14.
22. Njie YF, Qiao Z, Xiao Z, Wang W, Song ZH. N-Arachidonylethanolamide-induced increase in aqueous humor outflow facility. Invest Ophthalmol Vis Sci 2008;49:4528–34.
23. Sappington RM, Sidorova T, Long DJ, Calkins DJ. TRPV1: contribution to retinal ganglion cell apoptosis and increased intracellular Ca2+ with exposure to hydrostatic pressure. Invest Ophthalmol Vis Sci 2009;50:717–28.
24. Hu SS, Arnold A, Hutchens JM, Radicke J, Cravatt BF, Wager-Miller J, et al. Architecture of cannabinoid signaling in mouse retina. J Comp Neurol 2010;518:3848–66.
25. Lopez EM, Tagliaferro P, Onaivi ES, Lopez-Costa JJ. Distribution of CB2 cannabinoid receptor in adult rat retina. Synapse 2011;65:388–92.
26. Zabouri N, Bouchard JF, Casanova C. Cannabinoid receptor type 1 expression during postnatal development of the rat retina. J Comp Neurol 2011;519:1258–80.
27. Bouskila J, Burke MW, Zabouri N, Casanova C, Ptito M, Bouchard JF. Expression and localization of the cannabinoid receptor type 1 and the enzyme fatty acid amide hydrolase in the retina of vervet monkeys. Neuroscience 2012;202:117–30.
28. Kumar A, Qiao Z, Kumar P, Song ZH. Effects of palmitoylethanolamide on aqueous humor outflow. Invest Ophthalmol Vis Sci 2012;53:4416–25.
29. Romano MR, Lograno MD. Involvement of the peroxisome proliferator-activated receptor (PPAR) alpha in vascular response of endocannabinoids in the bovine ophthalmic artery. Eur J Pharmacol 2012;683:197–203.
30. Bouskila J, Javadi P, Casanova C, Ptito M, Bouchard JF. Muller cells express the cannabinoid CB2 receptor in the vervet monkey retina. J Comp Neurol 2013;521:2399–415.
31. Bouskila J, Javadi P, Casanova C, Ptito M, Bouchard JF. Rod photoreceptors express GPR55 in the adult vervet monkey retina. PLoS One 2013;8:e81080.
32. Caldwell MD, Hu SS, Viswanathan S, Bradshaw H, Kelly ME, Straiker A. A GPR18-based signalling system regulates IOP in murine eye. Br J Pharmacol 2013;169:834–43.
33. Cécyre B, Zabouri N, Huppe-Gourgues F, Bouchard JF, Casanova C. Roles of cannabinoid receptors type 1 and 2 on the retinal function of adult mice. Invest Ophthalmol Vis Sci 2013;54:8079–90.
34. MacIntyre J, Dong A, Straiker A, Zhu J, Howlett SE, Bagher A, et al. Cannabinoid and lipid-mediated vasorelaxation in retinal microvasculature. Eur J Pharmacol 2014;735:105–14.
35. Alexander SP, Kendall DA. The complications of promiscuity: endocannabinoid action and metabolism. Br J Pharmacol 2007;152:602–23.
36. Di Marzo V. Endocannabinoid signaling in the brain: biosynthetic mechanisms in the limelight. Nat Neurosci 2011;14:9–15.
37. Slusar JE, Cairns EA, Szczesniak AM, Bradshaw HB, Di Polo A, Kelly ME. The fatty acid amide hydrolase inhibitor, URB597, promotes retinal ganglion cell neuroprotection in a rat model of optic nerve axotomy. Neuropharmacology 2013;72C:116–25.
38. Murataeva N, Mackie K, Straiker A. The CB2-preferring agonist JWH015 also potently and efficaciously activates CB1 in autaptic hippocampal neurons. Pharmacol Res 2012;66:437–42.
39. Nucci C, Bari M, Spano A, Corasaniti M, Bagetta G, Maccarrone M, et al. Potential roles of (endo)cannabinoids in the treatment of glaucoma: from intraocular pressure control to neuroprotection. Prog Brain Res 2008;173:451–64.
40. Di Marzo V, Petrosino S. Endocannabinoids and the regulation of their levels in health and disease. Curr Opin Lipidol 2007;18:129–40.
41. Hepler RS, Frank IR. Marihuana smoking and intraocular pressure. J Am Med Assoc 1971;217:1392.
42. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol 2002;120:1268–79.
43. Schmidl D, Schmetterer L, Garhofer G, Popa-Cherecheanu A. Pharmacotherapy of glaucoma. J Ocul Pharmacol Ther 2015;31:63–77.
44. Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nat Rev Drug Discov 2012;11:541–59.
45. Tamm ER, Schmetterer L, Grehn F. Status and perspectives of neuroprotective therapies in glaucoma: the European Glaucoma Society White Paper. Cell Tissue Res 2013;353:347–54.
46. Civan MM. Chapter 1: Formation of the aqueous humor: transport components and their integration. In: Civan MM, editor. Current topics in membranes. San Diego, CA, USA: Academic Press, 2008:1–45.
47. Fautsch MP, Johnson DH. Aqueous humor outflow: what do we know? Where will it lead us? Invest Ophthalmol Vis Sci 2006;47:4181–7.
48. Merritt JC, Crawford WJ, Alexander PC, Anduze AL, Gelbart SS. Effect of marihuana on intraocular and blood pressure in glaucoma. Ophthalmology 1980;87:222–8.
49. Porcella A, Maxia C, Gessa GL, Pani L. The synthetic cannabinoid WIN55212-2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies. Eur J Neurosci 2001;13:409–12.
50. Chien FY, Wang RF, Mittag TW, Podos SM. Effect of WIN 55212-2, a cannabinoid receptor agonist, on aqueous humor dynamics in monkeys. Arch Ophthalmol 2003;121:87–90.
51. Szczesniak AM, Kelly ME, Whynot S, Shek PN, Hung O. Ocular hypotensive effects of an intratracheally delivered liposomal delta9-tetrahydrocannabinol preparation in rats. J Ocul Pharmacol Ther 2006;22:160–7.
52. Tomida I, Azuara-Blanco A, House H, Flint M, Pertwee RG, Robson PJ. Effect of sublingual application of cannabinoids on intraocular pressure: a pilot study. J Glaucoma 2006;15:349–53.
53. Hudson BD, Beazley M, Szczesniak AM, Straiker A, Kelly ME. Indirect sympatholytic actions at beta-adrenoceptors account for the ocular hypotensive actions of cannabinoid receptor agonists. J Pharmacol Exp Ther 2011;339:757–67.
54. Lograno MD, Romano MR. Cannabinoid agonists induce contractile responses through Gi/o-dependent activation of phospholipase C in the bovine ciliary muscle. Eur J Pharmacol 2004;494:55–62.
55. Bab I, Zimmer A. Cannabinoid receptors and the regulation of bone mass. Br J Pharmacol 2008;153:182–8.
56. Robson P. Therapeutic aspects of cannabis and cannabinoids. Br J Psychiatry 2001;178:107–15.
57. Qu J, Wang D, Grosskreutz CL. Mechanisms of retinal ganglion cell injury and defense in glaucoma. Exp Eye Res 2010;91:48–53.

58. Hara MR, Snyder SH. Cell signaling and neuronal death. Annu Rev Pharmacol Toxicol 2007;47:117–41.
59. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philos Trans R Soc Lond B Biol Sci 2012;367:3353–63.
60. Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview. Immunobiology 2010;215:588–97.
61. Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther 1995;273:734–43.
62. Karanian DA, Brown QB, Makriyannis A, Kosten TA, Bahr BA. Dual modulation of endocannabinoid transport and fatty acid amide hydrolase protects against excitotoxicity. J Neurosci 2005;25:7813–20.
63. Karanian DA, Karim SL, Wood JT, Williams JS, Lin S, Makriyannis A, et al. Endocannabinoid enhancement protects against kainic acid-induced seizures and associated brain damage. J Pharmacol Exp Ther 2007;322:1059–66.
64. Gomez del Pulgar T, Velasco G, Guzman M. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. Biochem J 2000;347(Pt 2):369–73.
65. Yune TY, Park HG, Lee JY, Oh TH. Estrogen-induced Bcl-2 expression after spinal cord injury is mediated through phosphoinositide-3-kinase/Akt-dependent CREB activation. J Neurotrauma 2008;25:1121–31.
66. Pignataro G, Meller R, Inoue K, Ordonez AN, Ashley MD, Xiong Z, et al. In vivo and in vitro characterization of a novel neuroprotective strategy for stroke: ischemic postconditioning. J Cereb Blood Flow Metab 2008;28:232–41.
67. Moranta D, Esteban S, Garcia-Sevilla JA. Acute, chronic and withdrawal effects of the cannabinoid receptor agonist WIN55212-2 on the sequential activation of MAPK/Raf-MEK-ERK signaling in the rat cerebral frontal cortex: short-term regulation by intrinsic and extrinsic pathways. J Neurosci Res 2007;85:656–67.
68. Jackson SJ, Diemel LT, Pryce G, Baker D. Cannabinoids and neuroprotection in CNS inflammatory disease. J Neurol Sci 2005;233:21–5.
69. Stefano GB, Liu Y, Goligorsky MS. Cannabinoid receptors are coupled to nitric oxide release in invertebrate immunocytes, microglia, and human monocytes. J Biol Chem 1996;271: 19238–42.
70. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia 2010;58:1017–30.
71. Waksman Y, Olson JM, Carlisle SJ, Cabral GA. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. J Pharmacol Exp Ther 1999;288:1357–66.
72. Walter L, Stella N. Cannabinoids and neuroinflammation. Br J Pharmacol 2004;141:775–85.
73. El-Remessy AB, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai NJ, Roon P, et al. Neuroprotective effect of (–)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol 2003;163:1997–2008.
74. Crandall J, Matragoon S, Khalifa YM, Borlongan C, Tsai NT, Caldwell RB, et al. Neuroprotective and intraocular pressure-lowering effects of (–)Delta9-tetrahydrocannabinol in a rat model of glaucoma. Ophthalmic Res 2007;39:69–75.
75. Pinar-Sueiro S, Zorrilla Hurtado JA, Veiga-Crespo P, Sharma SC, Vecino E. 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 2013;110:55–8.
76. Yoles E, Belkin M, Schwartz M. HU-211, a nonpsychotropic cannabinoid, produces short- and long-term neuroprotection after optic nerve axotomy. J Neurotrauma 1996;13:49–57.
77. Song ZH, Slowey CA. Involvement of cannabinoid receptors in the intraocular pressure-lowering effects of WIN55212-2. J Pharmacol Exp Ther 2000;292:136–9.
78. American Glaucoma Society. Position statement on marijuana and the treatment of glaucoma. August 10, 2009.
79. Buys YM, Rafuse P. Medical use of marijuana for glaucoma. Policy and position statement of the Canadian Ophthalmology Society (COS). April 2010.
80. Ross RA. Allosterism and cannabinoid CB(1) receptors: the shape of things to come. Trends Pharmacol Sci 2007;28:567–72.
81. Hudson BD, Hebert TE, Kelly ME. Ligand- and heterodimer-directed signaling of the CB(1) cannabinoid receptor. Mol Pharmacol 2010;77:1–9.
82. Di Marzo V. Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov 2008;7:438–55.
83. Kohnz RA, Nomura DK. Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids. Chem Soc Rev 2014;43:6859–69.
84. Pertwee RG. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J 2005;7:E625–54.
85. Pamplona FA, Ferreira J, Menezes de Lima O Jr., Duarte FS, Bento AF, Forner S, et al. Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. Proc Natl Acad Sci USA 2012;109:21134–9.
86. Tchantchou F, Zhang Y. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma 2013;30:565–79.
87. Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science 2011;334:809–13.
88. Fernandez-Suarez D, Celorrio M, Riezu-Boj JI, Ugarte A, Pacheco R, Gonzalez H, et al. The monoacylglycerol lipase inhibitor JZL184 is neuroprotective and alters glial cell phenotype in the chronic MPTP mouse model. Neurobiol Aging 2014;35:2603–16.
89. Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, et al. Allosteric modulation of the cannabinoid CB1 receptor. Mol Pharmacol 2005;68:1484–95.
90. Horswill JG, Bali U, Shaaban S, Keily JF, Jeevaratnam P, Babbs AJ, et al. PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. Br J Pharmacol 2007;152:805–14.
91. Navarro HA, Howard JL, Pollard GT, Carroll FI. Positive allosteric modulation of the human cannabinoid (CB) receptor by RTI-371, a selective inhibitor of the dopamine transporter. Br J Pharmacol 2009;156:1178–84.
92. Ahn KH, Mahmoud MM, Kendall DA. Allosteric modulator ORG27569 induces CB1 cannabinoid receptor high affinity agonist binding state, receptor internalization, and

Gi protein-independent ERK1/2 kinase activation. J Biol Chem 2012;287:12070–82.
93. Baillie GL, Horswill JG, Anavi-Goffer S, Reggio PH, Bolognini D, Abood ME, et al. CB(1) receptor allosteric modulators display both agonist and signaling pathway specificity. Mol Pharmacol 2013;83:322–38.
94. Wootten D, Christopoulos A, Sexton PM. Emerging paradigms in GPCR allostery: implications for drug discovery. Nat Rev Drug Discov 2013;12:630–44.
95. Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. J Mol Endocrinol 2010;44:75–85.
96. Ahn KH, Mahmoud MM, Shim JY, Kendall DA. Distinct roles of beta-arrestin 1 and beta-arrestin 2 in ORG27569-induced biased signaling and internalization of the cannabinoid receptor 1 (CB1). J Biol Chem 2013;288:9790–800.
97. Cawston EE, Redmond WJ, Breen CM, Grimsey NL, Connor M, Glass M. Real-time characterization of cannabinoid receptor 1 (CB1) allosteric modulators reveals novel mechanism of action. Br J Pharmacol 2013;170:893–907.
98. Luttrell LM. Minireview: more than just a hammer: ligand "bias" and pharmaceutical discovery. Mol Endocrinol 2014;28:281–94.
99. Ryskamp DA, Redmon S, Jo AO, Krizaj D. TRPV1 and endocannabinoids: emerging molecular signals that modulate mammalian vision. Cells 2014;3:914–38.
100. Leonelli M, Martins DO, Britto LR. TRPV1 receptors are involved in protein nitration and Muller cell reaction in the acutely axotomized rat retina. Exp Eye Res 2010;91:755–68.
101. Sakamoto K, Kuroki T, Okuno Y, Sekiya H, Watanabe A, Sagawa T, et al. Activation of the TRPV1 channel attenuates N-methyl-D-aspartic acid-induced neuronal injury in the rat retina. Eur J Pharmacol 2014;733:13–22.
102. Sappington RM, Sidorova T, Ward NJ, Chakravarthy R, Ho KW, Calkins DJ. Activation of transient receptor potential vanilloid-1 (TRPV1) influences how retinal ganglion cell neurons respond to pressure-related stress. Channels (Austin) 2015;9:102–13.
103. Ward NJ, Ho KW, Lambert WS, Weitlauf C, Calkins DJ. Absence of transient receptor potential vanilloid-1 accelerates stress-induced axonopathy in the optic projection. J Neurosci 2014;34:3161–70.
104. Kohno M, Hasegawa H, Inoue A, Muraoka M, Miyazaki T, Oka K, et al. Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem Biophys Res Commun 2006;347:827–32.
105. Szczesniak AM, Maor Y, Robertson H, Hung O, Kelly ME. Nonpsychotropic cannabinoids, abnormal cannabidiol and canabigerol-dimethyl heptyl, act at novel cannabinoid receptors to reduce intraocular pressure. J Ocul Pharmacol Ther 2011;27:427–35.
106. Mikawa Y, Matsuda S, Kanagawa T, Tajika T, Ueda N, Mimura Y. Ocular activity of topically administered anandamide in the rabbit. Jpn J Ophthalmol 1997;41:217–20.
107. Gagliano C, Ortisi E, Pulvirenti L, Reibaldi M, Scollo D, Amato R, et al. Ocular hypotensive effect of oral palmitoyl-ethanolamide: a clinical trial. Invest Ophthalmol Vis Sci 2011;52:6096–100.
108. Costagliola C, Romano MR, dell'Omo R, Russo A, Mastropasqua R, Semeraro F. Effect of palmitoylethanolamide on visual field damage progression in normal tension glaucoma patients: results of an open-label six-month follow-up. J Med Food 2014;17:949–54.
109. Esposito E, Cuzzocrea S. Palmitoylethanolamide in homeostatic and traumatic central nervous system injuries. CNS Neurol Disord Drug Targets 2013;12:55–61.
110. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.
111. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58:389–462.
112. Croxford JL, Yamamura T. Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? J Neuroimmunol 2005;166:3–18.
113. Xu H, Cheng CL, Chen M, Manivannan A, Cabay L, Pertwee RG, et al. Anti-inflammatory property of the cannabinoid receptor-2-selective agonist JWH-133 in a rodent model of autoimmune uveoretinitis. J Leukoc Biol 2007;82:532–41.
114. Toguri JT, Lehmann C, Laprairie RB, Szczesniak AM, Zhou J, Denovan-Wright EM, et al. Anti-inflammatory effects of cannabinoid CB(2) receptor activation in endotoxin-induced uveitis. Br J Pharmacol 2014;171:1448–61.
115. Toguri JT, Moxsom R, Szczesniak AM, Zhou J, Kelly ME, Lehmann C. Cannabinoid receptor 2 activation reduces leukocyte adhesion and improves capillary perfusion in the iridial microvasculature during systemic inflammation. Clin Hemorheol Microcirc 2015. DOI: 10.3233/CH-151996.
116. Ofek O, Karsak M, Leclerc N, Fogel M, Frenkel B, Wright K, et al. Peripheral cannabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci USA 2006;103:696–701.
117. Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995;232:54–61.
118. De Filippo K, Dudeck A, Hasenberg M, Nye E, van Rooijen N, Hartmann K, et al. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. Blood 2013;121:4930–7.
119. McHugh D, Tanner C, Mechoulam R, Pertwee RG, Ross RA. Inhibition of human neutrophil chemotaxis by endogenous cannabinoids and phytocannabinoids: evidence for a site distinct from CB1 and CB2. Mol Pharmacol 2008;73:441–50.
120. Rom S, Persidsky Y. Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. J Neuroimmune Pharmacol 2013;8:608–20.
121. Rajesh M, Mukhopadhyay P, Batkai S, Hasko G, Liaudet L, Huffman JW, et al. CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. Am J Physiol Heart Circ Physiol 2007;293:H2210–8.
122. Ramirez SH, Hasko J, Skuba A, Fan S, Dykstra H, McCormick R, et al. Activation of cannabinoid receptor 2 attenuates leukocyte-endothelial cell interactions and blood-brain barrier dysfunction under inflammatory conditions. J Neurosci 2012;32:4004–16.
123. Adhikary S, Kocieda VP, Yen JH, Tuma RF, Ganea D. Signaling through cannabinoid receptor 2 suppresses murine dendritic cell migration by inhibiting matrix metalloproteinase 9 expression. Blood 2012;120:3741–9.
124. Matias I, Pochard P, Orlando P, Salzet M, Pestel J, Di Marzo V. Presence and regulation of the endocannabinoid system in human dendritic cells. Eur J Biochem 2002;269:3771–8.

125. Berdyshev EV. Cannabinoid receptors and the regulation of immune response. Chem Phys Lipids 2000;108:169–90.
126. Basu S, Dittel BN. Unraveling the complexities of cannabinoid receptor 2 (CB2) immune regulation in health and disease. Immunol Res 2011;51:26–38.
127. Rom S, Zuluaga-Ramirez V, Dykstra H, Reichenbach NL, Pacher P, Persidsky Y. Selective activation of cannabinoid receptor 2 in leukocytes suppresses their engagement of the brain endothelium and protects the blood-brain barrier. Am J Pathol 2013;183:1548–58.
128. Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. Inflamm Bowel Dis 2009;15:1678–85.
129. Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem 2005;95:437–45.
130. Li S, Li B, Jiang H, Wang Y, Qu M, Duan H, et al. Macrophage depletion impairs corneal wound healing after autologous transplantation in mice. PLoS One 2013;8:e61799.
131. McMenamin PG, Crewe J, Morrison S, Holt PG. Immunomorphologic studies of macrophages and MHC class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 1994;35:3234–50.
132. Pouvreau I, Zech JC, Thillaye-Goldenberg B, Naud MC, Van Rooijen N, de Kozak Y. Effect of macrophage depletion by liposomes containing dichloromethylene-diphosphonate on endotoxin-induced uveitis. J Neuroimmunol 1998;86:171–81.
133. London A, Benhar I, Schwartz M. The retina as a window to the brain – from eye research to CNS disorders. Nat Rev Neurol 2013;9:44–53.
134. Taylor AW, Kaplan HJ. Ocular immune privilege in the year 2010: ocular immune privilege and uveitis. Ocul Immunol Inflamm 2010;18:488–92.
135. Mochizuki M, Sugita S, Kamoi K. Immunological homeostasis of the eye. Prog Retin Eye Res 2013;33:10–27.
136. Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J Leukoc Biol 2003;74:179–85.
137. Caspi RR. A look at autoimmunity and inflammation in the eye. J Clin Invest 2010;120:3073–83.
138. Caspi RR. Immune mechanisms in uveitis. Springer Semin Immunopathol 1999;21:113–24.
139. Larson T, Nussenblatt RB, Sen HN. Emerging drugs for uveitis. Expert Opin Emerg Drugs 2011;16:309–22.
140. LeHoang P. The gold standard of noninfectious uveitis: corticosteroids. Dev Ophthalmol 2012;51:7–28.
141. Servat JJ, Mears KA, Black EH, Huang JJ. Biological agents for the treatment of uveitis. Expert Opin Biol Ther 2012;12:311–28.
142. Altinsoy A, Dilekoz E, Kul O, Ilhan SO, Tunccan OG, Seven I, et al. A cannabinoid ligand, anandamide, exacerbates endotoxin-induced uveitis in rabbits. J Ocul Pharmacol Ther 2011;27:545–52.
143. Pryce G, Ahmed Z, Hankey DJ, Jackson SJ, Croxford JL, Pocock JM, et al. Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. Brain 2003;126(Pt 10):2191–202.
144. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. J Am Med Assoc 2007;298:902–16.
145. Cuenca N, Fernandez-Sanchez L, Campello L, Maneu V, De la Villa P, Lax P, et al. Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. Prog Retin Eye Res 2014;43:17–75.
146. El-Remessy AB, Al-Shabrawey M, Khalifa Y, Tsai NT, Caldwell RB, Liou GI. Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. Am J Pathol 2006;168:235–44.
147. Burstein S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. Bioorg Med Chem 2015;23:1377–85.
148. El-Remessy AB, Rajesh M, Mukhopadhyay P, Horvath B, Patel V, Al-Gayyar MM, et al. Cannabinoid 1 receptor activation contributes to vascular inflammation and cell death in a mouse model of diabetic retinopathy and a human retinal cell line. Diabetologia 2011;54:1567–78.
149. Wei Y, Wang X, Wang L. Presence and regulation of cannabinoid receptors in human retinal pigment epithelial cells. Mol Vis 2009;15:1243–51.
150. Wei Y, Wang X, Zhao F, Zhao PQ, Kang XL. Cannabinoid receptor 1 blockade protects human retinal pigment epithelial cells from oxidative injury. Mol Vis 2013;19:357–66.
151. Bereiter DA, Bereiter DF, Hirata H. Topical cannabinoid agonist, WIN55,212-2, reduces cornea-evoked trigeminal brainstem activity in the rat. Pain 2002;99:547–56.
152. Yang Y, Yang H, Wang Z, Varadaraj K, Kumari SS, Mergler S, et al. Cannabinoid receptor 1 suppresses transient receptor potential vanilloid 1-induced inflammatory responses to corneal injury. Cell Signal 2013;25:501–11.
153. Murataeva N, Li S, Oehler O, Miller S, Dhopeshwarkar A, Hu SS, et al. Cannabinoid-induced chemotaxis in bovine corneal epithelial cells. Invest Ophthalmol Vis Sci 2015;56:3304–13.