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Cannabinoids in the treatment of glaucoma

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Abstract

The leading cause of irreversible blindness is glaucoma, a disease normally characterized by the development of ocular hypertension and consequent damage to the optic nerve at its point of retinal attachment. This results in a narrowing of the visual field, and eventually results in blindness. A number of drugs are available to lower intraocular pressure (IOP), but, occasionally, they are ineffective or have intolerable side-effects for some patients and can lose efficacy with chronic administration. The smoking of marijuana has decreased IOP in glaucoma patients. Cannabinoid drugs, therefore, are thought to have significant potential for pharmaceutical development. However, as the mechanism surrounding their effect on IOP initially was thought to involve the CNS, issues of psychoactivity hindered progress. The discovery of ocular cannabinoid receptors implied an explanation for the induction of hypotension by topical cannabinoid applications, and has stimulated a new phase of ophthalmic cannabinoid research. Featured within these investigations is the possibility that at least some cannabinoids may ameliorate optic neuronal damage through suppression of *N*-methyl-D-aspartate receptor hyperexcitability, stimulation of neural microcirculation, and the suppression of both apoptosis and damaging free radical reactions, among other mechanisms. Separation of therapeutic actions from side-effects now seems possible through a diverse array of novel chemical, pharmacological, and formulation strategies.

Keywords: Cannabinoids; Endocannabinoids; CB receptor; Intraocular pressure; Glaucoma; Topical

Abbreviations: AEA, arachidonylethanolamide; 2-AG, 2-arachidonoylglycerol; CD, cyclodextrin; COX, cyclo-oxygenase; FAAH, fatty acid amide hydrolase; HP-β-CD, hydroxypropyl-β-cyclodextrin; IOP, intraocular pressure; NMDA, *N*-methyl-D-aspartate; P_{app}, apparent partition coefficient; PMSF, phenylmethylsulfonyl fluoride; SBE-β-CD, sulfobutylether-β-cyclodextrin; THC, tetrahydrocannabinol; VR1, vanilloid receptor Type 1.

Contents

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1. Introduction

Glaucoma is the leading cause of irreversible blindness, and it is estimated that 66.8 million people are presently affected, of whom, 6.7 million will become blind in both eyes (Alward, 1998). Current glaucoma treatment includes α_2-adrenoreceptor agonists, β-adrenoreceptor agonists, dopaminergic agonists, cholinergic agonists, carbonic anhydrase inhibitors, and prostaglandin agonists, all of which are ocular hypotensive agents (Sugrue, 1997).

Hepler and Frank (1971) originally observed that subjects who smoked marijuana developed a reduced intraocular pressure (IOP). Subsequent human experiments involving oral (Merritt et al., 1980b) and intravenous (Cooler & Gregg, 1977) administration of pure Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient in *Cannabis sativa* L., confirmed this observation and pinpointed the active component. As a result, a great deal of research exploring this compound, and related compounds, as a possible glaucoma drug(s) has been published over the last 20 years (e.g., Green, 1998, 2000; Green & McDonald, 1987). However, clinical application of Δ^9-THC may include adverse psychic or somatic side-effects, in addition to the fact that the lipophilic cannabinoids are insoluble in water, hindering their use as topical agents.

Discovery of a cannabinoid receptor (Devane et al., 1988) and its endogenous ligand (Devane et al., 1992b), arachidonylethanolamide (AEA), offered new horizons for the use of cannabinoids in medicine, including glaucoma therapy. The presence of CB_1 receptors (Porcella et al., 1998; Straiker et al., 1999a, 1999b) and an AEA-specific enzyme activity in the eye (Matsuda et al., 1997) provided the context for a mechanism of drug action. In addition, novel formulation technologies, such as cyclodextrins (CDs) (Jarho et al., 1998) or microemulsions (Muchtar et al., 1992), enable the preparation of topical dosage forms for these highly lipophilic cannabinoids. Topically administered cannabinoids having optimal ophthalmic delivery properties would minimize drug concentrations in systemic circulation and possible consequent adverse side-effects.

This review will focus on the cannabinoids as a potential class of topical anti-glaucoma agents, apparently acting upon a newly discovered ocular cannabinoid receptor to lower the ocular hypertension symptomatic of the disease. In addition, mention will be made of possible complimentary mechanisms by which this class of drugs may also offer unique advantages for ameliorating the collateral neurodegenerative effects of this disease. In addition, a brief summary concerning the main features of drug delivery after ocular administration and CD technology in ophthalmic applications is described in order to clarify future challenges in the research and development of novel ophthalmic cannabinoids.

2. Ocular drug delivery

Topical delivery of eye drops into the lower cul-de-sac of the eye is the most common method of drug treatment in ocular disease. In general, the site of action for ophthalmic drugs is located inside the eye. Unfortunately, after instillation of an eyedrop, typically $< 5\%$ of an applied dose reaches the intraocular tissues. The main reason of this low ocular drug availability is poor drug penetration across the

corneal barrier and a rapid loss of the instilled solution from the pre-corneal area. Ocular absorption and pharmacokinetics have been described in numerous reviews (e.g., Davies, 2000; Järvinen, K. et al., 1995; Sasaki et al., 1999). Only the most salient features of ophthalmic drug delivery are summarized herein to help readers understand the process of drug development, especially as applied to cannabinoids.

2.1. Pre-corneal drug elimination

After topical administration, aqueous eyedrop solutions mix with tear fluid and are dispersed over the eye surface. However, various pre-corneal factors (i.e., drainage of instilled solution, non-corneal absorption, induced lacrimation) limit ocular absorption by shortening the cornea contact time of applied drugs. These factors, and the corneal barrier itself, limit penetration of a topically administered ophthalmic drug. As a result, only a few percent of the applied dose is delivered into the intraocular tissues, the major part (50–99%) being absorbed into the systemic circulation (Fig. 1), where it can cause various side-effects.

The main sites for systemic absorption are the nasal mucosa and the ocular conjunctiva (Urtti & Salminen, 1993). Following instillation of an applied eyedrop (25–50 μL) onto the pre-corneal area of the eye, the greater part of the drug solution rapidly exits the eye surface via the lacrimal drainage system, nasal mucosa, and pharynx. Soon thereafter, the resident tear volume of 7.5 μL returns to normal (Chrai et al., 1973). Compared with the cornea, the conjunctiva is highly vascularized, with a 10-fold greater surface area (Watsky et al., 1988). It is also 2–30 times more permeable, depending on the administered drug (Ahmed et al., 1987; Wang et al., 1991).

2.2. Corneal barriers

The cornea is generally considered to be a major, but not exclusive, pathway for the ocular penetration of topically applied drugs (Doane et al., 1978). Compared with many other epithelial tissues (e.g., bronchial, intestinal, nasal, tracheal), the corneal epithelium is relatively impermeable, but less so than the stratum corneum of the skin (Rojanasakul et al., 1992). Although the cornea is composed of five layers, the epithelium and the stroma are most significant for drug delivery (Huang et al., 1983). The lipophilic epithelium is the primary barrier for corneal permeation by highly hydrophilic drugs. However, for highly lipophilic drugs, partitioning from the epithelium to the hydrophilic stroma is the rate-limiting step.

The apparent corneal permeability coefficient (or flux) is usually determined using an isolated cornea mounted in a side-by-side diffusion cell. In vitro corneal permeability studies produce information about the effects of drug structure and formulation on corneal permeability. However, in vitro corneal permeability studies do not include pre-corneal loss processes and, therefore, do not reliably predict the in vivo bioavailability of topically administered drugs.

2.3. Physicochemical properties

Lipophilic drugs penetrate the corneal epithelium via the transcellular pathway and hydrophilic molecules utilize the paracellular route, the latter involving passive or altered

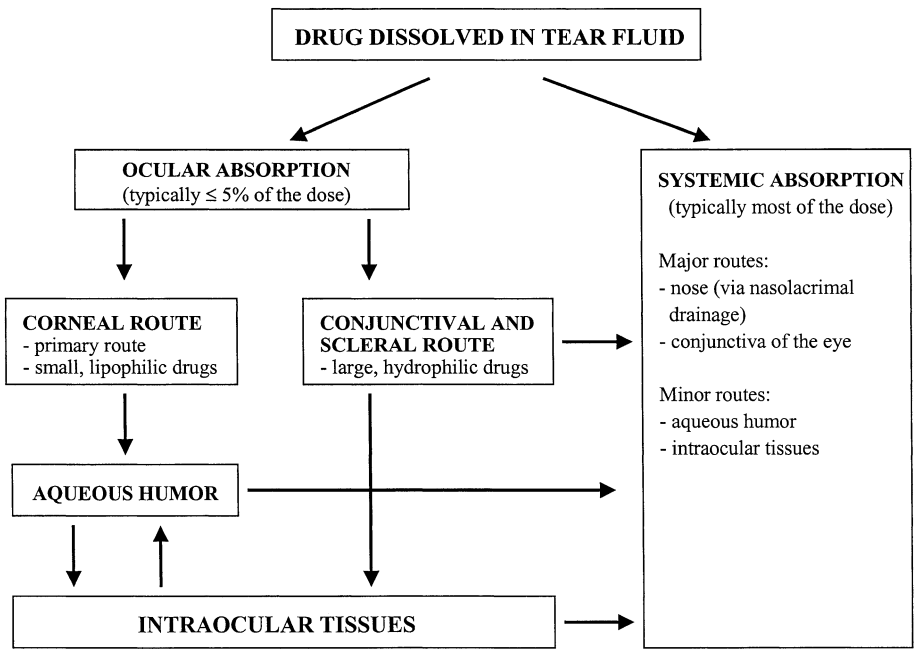

Fig. 1. Main absorption routes of a topically applied ophthalmic drug.

diffusion through intercellular spaces (Borchardt, 1990). For most topically applied drugs, this passive diffusion along a concentration gradient, which is largely influenced by various physicochemical properties, is the main corneal permeation mechanism.

Drug lipophilicity seems to be the most important property for corneal penetration, and both parabolic (Chien et al., 1991) and sigmoidal (Wang et al., 1991) curves have been used to describe their relationship. The optimum apparent partition coefficient (P_{app}; octanol/pH 7.4 buffer) for corneal drug absorption is in the range of 100–1000 (Schoenwald & Huang, 1983; Schoenwald & Ward, 1978), which is consistent with the lipophilic nature of the corneal epithelium (Sasaki et al., 1999).

Aqueous solubility is another drug property important for efficacy of delivery. The surface of the eye is constantly being cleaned and moistened by the aqueous tear fluid. Thus, it is difficult for drug molecules to be absorbed by the corneal epithelium, unless they are soluble in the tear film (Loftsson & Stefansson, 1997). In addition, the water solubility of the drug must be good enough to enable the formulation of aqueous eyedrops. The dilemma is that an ideal potential ophthalmic drug should simultaneously be both water-soluble and lipid soluble, but only a few molecules can fulfill these criteria. Because of that fact, various pharmaceutical technologies, such as CDs (Loftsson & Järvinen, 1999) and prodrugs (Järvinen & Järvinen, 1996), have been applied to improve the physicochemical properties of ophthalmic drugs. They are also useful approaches for the development of ophthalmic cannabinoids.

In addition to the lipophilicity and aqueous solubility of a drug, molecular size (Liaw & Robinson, 1992), charge (Liaw et al., 1992), and degree of ionization (Maren & Jankowska, 1985; Brechue & Maren, 1993) also affect corneal absorption. Tear fluid has a limited buffering capacity (Carney & Hill, 1979). Thus, pH and buffering capacity of the instilled solution affect the pH of the tear fluid and, consequently, drug ionization on the pre-corneal area. The non-ionized form of the drug usually penetrates the cornea more easily than the ionized form, so the pH and buffering capacity of an instilled solution can have a significant effect on ophthalmic drug absorption.

3. Cyclodextrins for ophthalmic drug delivery

3.1. Structure and function

CDs are macrocyclic oligosaccharides containing D-(+)-glucopyranose subunits joined through α-1,4-bonds (Fig. 2a). The most common natural CDs are α-CD, β-CD, and γ-CD, which contain six, seven, and eight glucose molecules, respectively. Each CD has varying inclusion capabilities that stem from differences in their internal cavity dimensions (Stella et al., 1999; Thompson, 1997).

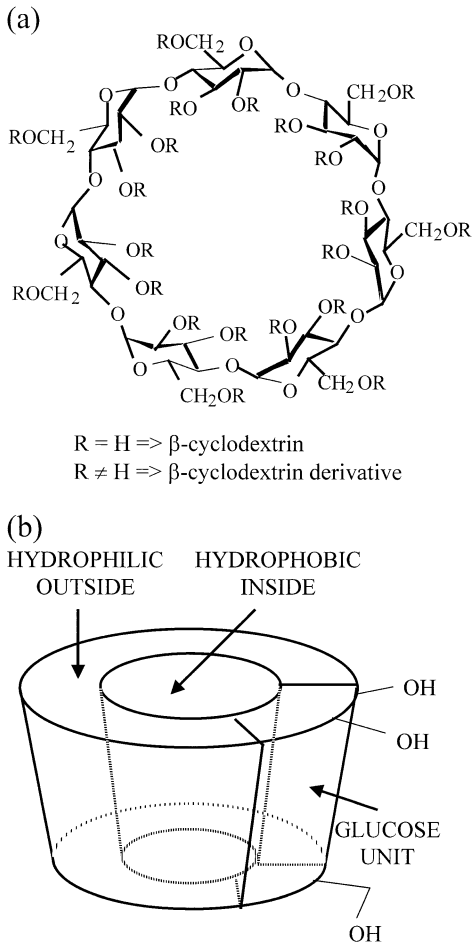

Fig. 2. Chemical structure (**a**) and the molecular shape (**b**) of β-CD.

CDs are cone-shaped molecules that are open at both ends (Fig. 2b). All their hydrophilic hydroxy groups are located on the outside of this structure, and the inside is relatively hydrophobic. These external OH groups can be chemically altered to produce CD derivatives with modified properties. Various CD derivatives have been developed to increase the aqueous solubility and safety of natural CDs, especially β-CD. Currently, the CD derivatives that are expected to have commercial pharmaceutic utility are randomly methylated derivatives of β-CD, 2-hydroxypropyl derivatives of β-CD (HP-β-CD), and a sulfobutylether derivative of β-CD (SBE-β-CD) (Thompson, 1997; Szente & Szejtli, 1999).

The most important feature of a CD is its ability to act as a "host" in the formation of inclusion complexes with hydrophobic "guest" molecules. An inclusion complex is formed when the guest molecule, or part of this molecule, enters into the hydrophobic cavity of the host CD. This complex is not a static entity, but a dynamic relationship between host and guest, and consists of the free drug, the drug/CD complex, and the unoccupied CD in an equilibrium that is dependent on several factors (Frömming & Szejtli, 1994; Szejtli, 1988). Complex formation depends on the

size, shape, and polarity of the guest molecule and on the properties of the CD that is used. Complexation can be used to increase the aqueous solubility, dissolution rate, stability, and bioavailability of drugs, as well as to modify the local irritation potential and organoleptic properties of therapeutic agents (Loftsson, 1995; Loftsson & Brewster, 1996; Rajewski & Stella, 1996; Zhang & Rees, 1999).

The safety profiles of natural CDs and their most commonly used derivatives have been reviewed recently (Irie & Uekama, 1997; Thompson, 1997). In general, natural CDs and most derivatives are unable to penetrate biological membranes. Thus, orally administered CDs are practically nontoxic. Furthermore, safety evaluations have shown that at least γ-CD, HP-β-CD, and SBE-β-CD appear to be safe, even when administered parenterally. However, α-CD, β-CD, and the methylated CDs are not suitable for parenteral administration.

3.2. Ophthalmic applications

In ophthalmology, CDs mainly have been used to improve the delivery of poorly water-soluble drugs (Loftsson & Järvinen, 1999; Loftsson & Stefansson, 1997). As pointed out by Rajewski and Stella (1996), poorly water-soluble drugs are frequently administered as suspensions or ointment-dosage forms, which may cause eye irritation and blurred vision, respectively. In addition, this limited water solubility hinders drug dissolution into tear fluid on the pre-corneal area and results in poor ophthalmic bioavailability (Bary et al., 2000; Fridriksdottir et al., 1997; Kristinsson et al., 1996).

Ophthalmic administration of CDs reveals some basic differences compared with the use of CDs via other routes of drug administration (Järvinen, K. et al., 1995; Rajewski & Stella, 1996). As it is generally assumed that only the free drug, and not the drug/CD complex, can penetrate across biological barriers, its release from this inclusion complex must take place before absorption can occur (Nakanishi et al., 1989; Frijlink et al., 1990). In contrast to oral and parenteral administration, ophthalmic preparations do not exhibit a significant increase in the fraction of free drug available after ocular administration. This is due to a lack of drug/CD-complex dilution by the small tear fluid volume ($\approx$ 7 μL). In addition, aqueous eyedrops are removed from the pre-corneal area within a few minutes (Chrai et al., 1973), and, thus, the drug/CD complex may not have enough time to release an effective dose of the drug before its clearance from the pre-corneal area. Especially in ophthalmic applications, excessive complexation of a drug may decrease its bioavailability (Bary et al., 2000; Davies et al., 1997; Järvinen, T. et al., 1995; Reer et al., 1994), although this drawback may be overcome by increasing the viscosity of the aqueous eyedrop formulation (Jarho et al., 1996a). Fig. 3 shows the kinetic parameters assumed to be critical for ophthalmic preparations containing CDs.

Studies dealing with the use of CDs in ophthalmic formulations usually involve in vitro (rarely in vivo) absorption studies. The in vitro studies are usually performed by monitoring the flux of a drug through semi-permeable membranes (Loftsson et al., 1994b) or the isolated corneas of experimental animals (Bary et al., 2000; Kearse & Green, 2000; Siefert et al., 1999). Both in vitro and in vivo studies have shown that the complexation of poorly water-soluble drugs with CDs increases corneal penetration/absorption of the drug compared with a suspension of the compound or a control preparation (Loftsson et al., 1994a; Reer et al., 1994; Siefert et al., 1999). This improved penetration/absorption is due to a fast dissociation of the drug/CD complex in solution, compared with the slow dissolution of a solid-drug suspension.

The interaction of CDs with cell membranes is a potential mechanism for increasing the bioavailability of ocular drugs

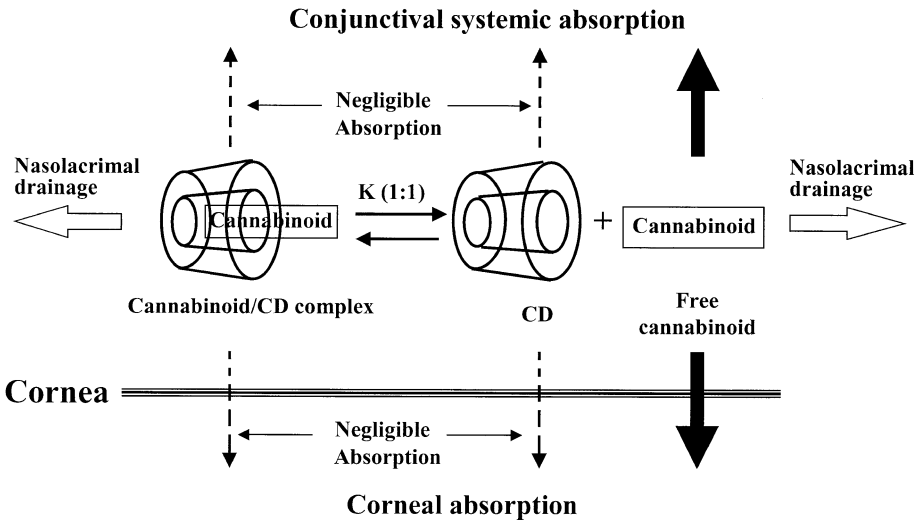

Fig. 3. Basic kinetic processes assumed to be critical in ophthalmic drug delivery in the presence of CDs.

(Reddy et al., 1996). In ophthalmic drug delivery, the most commonly studied CDs (HP-β-CD and SBE-β-CD) do not cause damage to corneal tissues (Jansen et al., 1990; Järvinen et al., 1994). Although pretreatment of the cornea with HP-β-CD and SBE-β-CD does not affect in vitro corneal penetration of water-soluble pilocarpine (Järvinen et al., 1994; Jarho et al., 1997), HP-β-CD (Freedman et al., 1993) and α-CD (Keipert et al., 1996) have been reported to increase its miotic effect. In addition, α-CD has been shown to improve the corneal penetration of pilocarpine due to CD-induced membrane effects (Siefert & Keipert, 1997). Methylated CDs are not commonly used in ophthalmic studies due to their damaging effect on the cornea (Jansen et al., 1990).

3.3. Cannabinoid administration

Cannabinoids have very poor aqueous solubility, which hinders their use in topical ophthalmic preparations. CDs are able to increase, by several orders of magnitude, the aqueous solubility of either AEA and its derivatives (Jarho et al., 1996b) or Δ^9-THC (Jarho et al., 1998). In addition, CDs significantly improve the aqueous stability of AEA (Jarho et al., 1996b). Thus, CDs would seem ideal as potential pharmaceutical excipients for the formulation of ophthalmic cannabinoids.

Fig. 4 shows how increasing CD concentrations affect the transcorneal flux of AEA solutions. With AEA mostly in suspension, its flux increases with greater CD solution strength, making an increase of AEA complexation possible. However, when all the AEA molecules have already been dissolved, this flux decreases at still greater CD concentrations due to excess drug/CD complexation and thus, a decreased concentration of free AEA in the donor phase. This study, and other similar studies through skin (Sigurdardottir & Loftsson, 1995) or nasal mucosa (Kublik et al., 1996), demonstrates the importance of an optimal CD concentration for ophthalmic applications. Recently, Kearse and Green (2000) reported the effect of various vehicles upon the in vitro transcorneal penetration of Δ^9-THC. Various vehicles were compared with the light mineral oil generally studied as a vehicle for topical Δ^9-THC applications, and α-CD showed significantly enhanced flux and penetration values. Interestingly, β-CD, γ-CD, and HP-β-CD did not significantly enhance flux and permeability compared with light mineral oil. However, the employed concentrations of γ-CD (20%) and HP-β-CD (30%) were too high, which may explain their modest flux values. The enhanced Δ^9-THC transcorneal flux shown with the α-CD vehicle is most probably due to the corneal effects of the latter (Siefert & Keipert, 1997).

4. Ocular cannabinoid systems

4.1. Endogenous cannabinoids

Two endogenous cannabinoid receptor ligands, AEA and 2-arachidonoylglycerol (2-AG) have been isolated from both nervous and peripheral tissues (Devane et al., 1992b; Mechoulam et al., 1995; Sugiura et al., 1995; Felder et al., 1996). The endogenous cannabinoids mimic the actions of Δ^9-THC, but are inactivated rapidly in vivo or in vitro. The inactivation of AEA or 2-AG occurs through a carrier-mediated cellular uptake process, followed by enzymatic hydrolysis, in cultured brain neurons and astrocytoma cells (Di Marzo et al., 1994; Beltramo & Piomelli, 2000; Bisogno et al., 2001). In cells, AEA is hydrolyzed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) (Deutsch & Chin, 1993; Cravatt et al., 1996; Ueda et al., 1995). The enzymatic hydrolysis of 2-AG to arachidonic acid and glycerol presumably is catalyzed by a yet unchar-

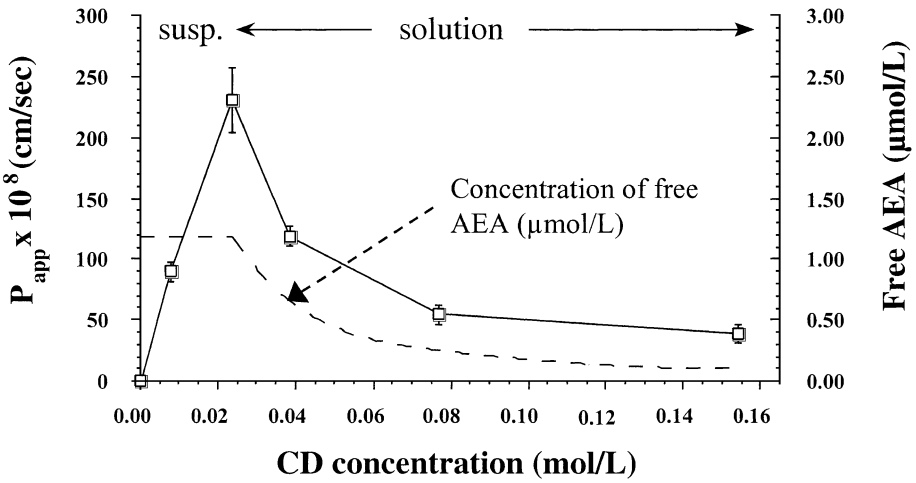

Fig. 4. Permeability (P_{app}, mean ± S.E., $n = 2–6$) of AEA through the isolated rabbit cornea as a function of HP-β-CD concentration (solid line) and calculated concentration of free AEA on the donor side as a function of HP-β-CD concentration (broken line). susp., suspension. Modified from Jarho et al. (1996b).

acterized monoacylglycerol lipase activity (Beltramo & Piomelli, 2000). Di Marzo et al. (1998b) have suggested that FAAH may be one of the enzymes deputed to the physiological inactivation of 2-AG. However, the contribution of FAAH to hydrolysis of 2-AG seems to be minor (Goparaju et al., 1999). The properties and the possible physiological roles of AEA and 2-AG have been discussed comprehensively in earlier reviews (Mechoulam et al., 1998; Piomelli et al., 2000; Bisogno et al., 2001).

The hydrolysis and biosynthesis of AEA in various porcine ocular tissues, including retina, iris, choroid, lacrimal gland, and optic nerve, has been reported (Matsuda et al., 1997). Both AEA synthase activity (1.9–4.2 nmol/min^{-1}/mg^{-1} protein at 37°C) and hydrolase activity (1.2–3.5 nmol/min^{-1}/mg^{-1} protein at 37°C) in the ocular tissues were comparable with those of brain homogenate. The highest enzyme activity was in the retina. However, condensation of AEA from arachidonic acid and ethanolamine under physiological conditions is unlikely, as the required arachidonic acid and ethanolamine concentrations are high (for recombinant rat liver FAAH, K_m values are 190 μM and 36 mM for arachidonic acid and ethanolamine, respectively) (Ueda et al., 1995; Kurahashi et al., 1997). Another biosynthetic pathway for AEA through phosphodiesterase-mediated cleavage of *N*-arachidonoylphosphatidylethanolamine has been suggested in cultured brain neurons (Di Marzo et al., 1994). The bovine retina has been shown to contain AEA and *N*-docosahexanoylethanolamine and their putative direct biosynthetic precursors, *N*-arachidonoylphosphatidylethanolamine and *N*-docosahexanoylphosphatidylethanolamine (Bisogno et al., 1999). In addition, FAAH-like activity that is inhibitable by the FAAH inhibitors phenylmethylsulfonyl fluoride (PMSF) and arachidonoyltrifluoromethylketone has also been identified in bovine retina (Bisogno et al., 1999), and an FAAH enzyme protein has been localized from rat retina using immunocytochemical methods (Yazulla et al., 1999).

Both endogenous cannabinoids AEA and 2-AG have been identified from bovine and rat retinas (Bisogno et al., 1999; Straiker et al., 1999b). The amounts of AEA and 2-AG in the bovine retina determined by gas chromatography-electron impact mass spectrometry were 64.0 ± 9.6 pmol/g and 1.63 ± 0.31 nmol/g of retinal tissue, respectively (Bisogno et al., 1999). The bovine retina contained 25 times more 2-AG than AEA. In rat retina, 2-AG (2.97 ± 0.066 nmol/g) was found in amounts similar to that of the brain (Straiker et al., 1999b), but AEA was not detectable. Other endogenous cannabinoid-like lipids, such as *N*-palmitoylethanolamide (a CB_2 receptor agonist) and *N*-oleoylethanolamine, were also identified from rat retina (Straiker et al., 1999b).

4.2. Cannabinoid receptors

Two cannabinoid receptor subtypes have been characterized from human and animal tissues (Devane et al., 1988; Munro et al., 1993). The CB_1 receptor is distributed throughout the CNS, whereas the CB_2 receptor is localized predominantly in peripheral tissues. Both cannabinoid receptors belong to the G-protein-coupled receptor superfamily.

The first indirect evidence for the possible existence of ocular cannabinoid receptors was provided in 1996 when Schlicker et al. inhibited dopamine release in guinea pig retinal discs by an application of the CB_1 receptor agonists WIN-55,212-2 and CP-55,940. The effect was reversed with the CB_1 receptor antagonist SR 141716A. This study was followed by the observation that metabolically stable forms of AEA (Pate et al., 1997, 1998) and CP-55,940 (Pate et al., 1998) lowered IOP in rabbits, an effect that was eliminated for either type compound upon subcutaneous pretreatment of the animals with SR 141716A (Pate et al., 1998). Subsequently, CB_1 receptor mRNA was identified in various ocular tissues of the rat (Porcella et al., 1998). CB_1 receptor mRNA is more abundant in the ciliary body area and iris than in the retina and choroid. In the human eye, most of the CB_1 receptor mRNA also appears to be in the ciliary body (Porcella et al., 2000).

By the use of subtype-specific affinity-purified polyclonal antibodies against the CB_1 receptor protein, a wide distribution of CB_1 receptors has been determined within the human anterior eye and retina (Straiker et al., 1999a). CB_1 receptors are present in the human ciliary epithelium, corneal epithelium and endothelium, trabecular meshwork, Canal of Schlemm, ciliary muscle, and in blood vessels of the ciliary body. In addition, retinal tissues of humans (Straiker et al., 1999a) and of several animal species (Straiker et al., 1999b; Yazulla et al., 1999, 2000) have been shown to contain CB_1 receptors. Evidence for the expression of CB_2 receptor mRNA has been obtained from rat retinal tissues by the use of in situ hybridization histochemistry and reverse transcription polymerase chain reaction (Lu et al., 2000).

The wide existence of cannabinoid receptors (Table 1) and their endogenous ligands in various eye tissues suggests a physiological role for the cannabinoid system in various ocular functions (Porcella et al., 1998; Straiker et al., 1999a; Lu et al., 2000). Endogenous cannabinoids acting via cannabinoid receptors may have effect, for example, on aqueous humor production and outflow, as well as on vision itself. Identification of the ocular cannabinoid system components enables the development of novel drugs that act specifically via the cannabinoid receptors of the eye.

5. Cannabinoids and intraocular pressure

Cannabinoids are generally classified according to their various chemical structures into the following four main groups: (1) classical cannabinoids (i.e., phytocannabinoids and synthetic congeners), (2) nonclassical cannabinoids (e.g., bicyclic compounds or nitrogen isosters), (3) amino-

Table 1
Regional distribution of cannabinoid receptors in the eye

Eye tissue	CB_1	CB_2	Species	Reference
Retina	X, Y	Y	Human, rat, mouse, monkey, goldfish, chick	Straiker et al., 1999a, 1999b; Yazulla et al., 1999, 2000; Lu et al., 2000; Porcella et al., 2000
Ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, photoreceptors				
Cornea	X		Human	Straiker et al., 1999a
Epithelium, endothelium				
Iris	X, Y		Human, rat	Porcella et al., 1998, 2000; Straiker et al., 1999a
Trabecular meshwork	X		Human	Straiker et al., 1999a
Schlemm's Canal	X		Human	Straiker et al., 1999a
Ciliary Body	X, Y		Human, rat	Porcella et al., 1998, 2000; Straiker et al., 1999a
Non-pigmented epithelium, muscle fibers				
Choroid	Y		Rat	Porcella et al., 1998

X, expression of cannabinoid receptor protein; Y, expression of cannabinoid receptor mRNA.

alkylindoles, and (4) eicosanoids (i.e., endocannabinoid congeners and related enzyme inhibitors). The chemical structures of prototypic members of each group are shown in Figs. 5–7.

5.1. Classical cannabinoids

Classical cannabinoids retain the various natural cannabinoid ring structures with their oxygen atoms. Typical of this class are the phytocannabinoids Δ^9-THC, cannabidiol, and cannabinol and the commercial synthetic drugs synhexyl and nabilone (Fig. 5). Hepler and Frank (1971) first published that smoked marijuana reduces IOP. As a result, several animal and human studies involving systemic and topical administration of Δ^9-THC or other classical cannabinoids have been carried out. Although HU-211 belongs to this taxon, its IOP effects, as discussed in Section 5.5, are not due to CB_1 receptor interactions.

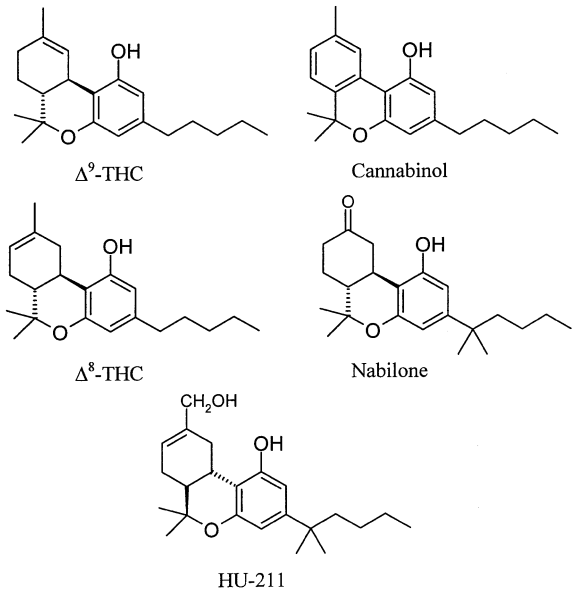

Fig. 5. Chemical structures of typical classical cannabinoids.

5.1.1. Systemic administration

Intravenous (ElSohly et al., 1981, 1984; Green et al., 1983) and oral (Howes, 1984; Merritt et al., 1980b; Waller et al., 1984) administration of various cannabinoids, including Δ^9-THC, Δ^8-THC, nabilone, and cannabinol, reduces IOP in animals and humans. However, it soon became apparent from the human studies that systemic routes of administration carried the burden of such undesirable side-effects as psychoactivity, conjunctival hyperemia, and postural hypotension (Green & McDonald, 1987), and, thus, more effort has been focused on studies with ophthalmic administration.

5.1.2. Ophthalmic administration

Topical application of cannabinoids to the eye seems to be the obvious alternative for minimizing systemic drug concentrations and maximizing the dose at the site of action. However, the hydrophobic cannabinoids have remarkably poor water solubility (Garrett & Hunt, 1974), so investigations employing several more physically compatible vehicles have been made, including sesame oil (Green & Bowman, 1976; Green et al., 1977, 1978) or mineral oil (Green et al., 1977, 1978; Merritt et al., 1981; Jay & Green, 1983), the latter of which worked best. Unfortunately, this vehicle can be irritating (Jay & Green, 1983) and has been demonstrated to influence IOP itself (Merritt et al., 1986).

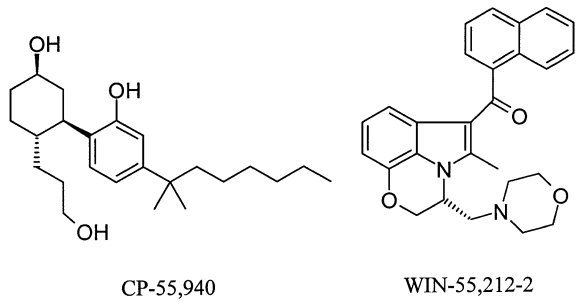

Fig. 6. Chemical structure of CP-55,940 (nonclassical cannabinoid) and WIN-55,212-2 (aminoalkylindole).

Colasanti et al. (1984a, 1984b, 1984c) employed polyethylene glycol for acute doses, as well as chronic administration via osmotic pumps. Use of cannabinoids in aqueous media with the aid of Tween 80 (Green et al., 1977), submicron aqueous emulsification (Muchtar et al., 1992), and CD complexes (Arsenovic, 1997; Jarho et al., 1998) have yielded various degrees of success.

Such research reveals that certain cannabinoids, after topical administration, are more efficacious than others, but that there remains a large degree of variability between these studies (Colasanti, 1986; Green, 1998, 2000). Much of this difference can be blamed upon vehicle choice and the fact that ocular absorption is very poor (Green et al., 1977) and probably inconsistent. The vast majority of drug not locally absorbed is available for systemic circulation (Chiang et al., 1983), and may affect other parts of the body.

5.2. Nonclassical cannabinoids

Nonclassical cannabinoids include bicyclic analogs of Δ^9-THC that lack a pyran "B" ring (Johnson & Melvin, 1986). The most important member of this group is CP-55,940 (Fig. 6), a compound that is broadly used in cannabinoid receptor-binding studies (Pertwee, 1997).

The topical application of CP-55,940 significantly decreases IOP in normotensive rabbits (Pate et al., 1998) and in those with elevated IOP (Sugrue et al., 1996). The unilateral ocular administration of CP-55,940 did not cause a decrease of IOP in untreated eyes equal to that of treated eyes, which suggests that the locus of action may be the eye rather than the CNS. The IOP decrease was neutralized by a subcutaneous pretreatment with SR 141716 (2.5 mg/kg), which suggests CB_1 receptor involvement with local IOP effects (Pate et al., 1998).

5.3. Aminoalkylindoles

Aminoalkylindoles form one important class of cannabinoids, the prototype molecule being WIN-55,212-2 (Fig. 6). WIN-55,212-2 binds to both the CB_1 and CB_2 receptors, but has a several-fold bias toward the CB_2 receptor (Felder & Glass, 1998; Pertwee, 1997; Showalter et al., 1996) and exhibits cannabinoid-like activity both in vitro and in vivo (Compton et al., 1992). WIN-55,212-3, the enantiomer of WIN-55,212-2, is inactive in both cannabinoid receptor systems (Compton et al., 1992).

Sugrue et al. (1996) first reported that topical administration of WIN-55,212-2 significantly decreases IOP in rabbits and monkeys, but that WIN-55,212-3 is significantly less active. The study also showed that a topical dose of WIN-55,212-2 decreased aqueous humor inflow 66%, whereas outflow was unchanged in the rabbits.

Hodges et al. (1997) reported that intravenous injection of WIN-55,212-2 (3 mg/kg) and of another cannabinoid did not cause a statistically significant IOP decrease in rabbits. However, they did mention that observed IOPs tended to decrease more substantially in some animals than in others.

Song and Slowey (2000) demonstrated that topical application of WIN-55,212-2 (dissolved in HP-β-CD) significantly decreased IOP in the treated eyes of rabbits, but no significant IOP reduction was observed in contralateral eyes. The maximal IOP reduction by 100 μg of WIN-55,212-2 was 4.7 ± 0.5 mm Hg at 2 hr after topical application. A topical dose (25 μg) of the CB_1 receptor antagonist SR141716A significantly attenuated the IOP effect of 100-μg WIN-55,212-2, which supports the results of Pate et al. (1998) and their hypothesis that a CB_1 receptor is involved in the IOP reduction effects of cannabinoid agonists. The inactive WIN-55,212-3 enantiomer did not decrease IOP.

Recently, Porcella et al. (2001) reported that a topical dose of WIN-55,212-2 (25 or 50 μg dissolved in HP-β-CD) significantly decreased the IOP of human glaucoma patients

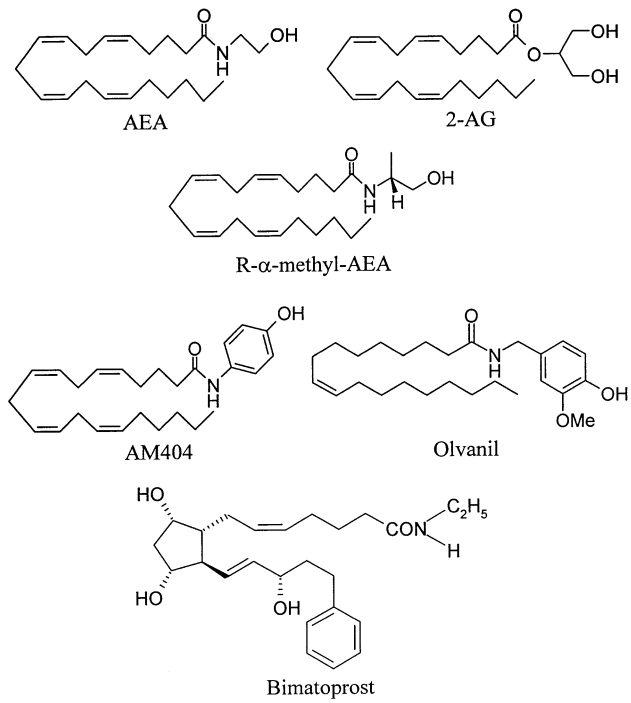

Fig. 7. Chemical structures of typical endocannabinoids and their congeners.

whose symptoms were refractory to conventional glaucoma therapy.

5.4. Endocannabinoids and their congeners

5.4.1. Arachidonylethanolamide and 2-arachidonoylglycerol

Topical administration of AEA (Fig. 7) (Mikawa et al., 1997; Pate et al., 1995) and 2-AG (Fig. 7) (Pate et al., 1996) to normotensive rabbits caused an initial increase and subsequent decrease in IOP in treated eyes. In untreated eyes, only a very weak IOP decrease (Pate et al., 1995) or no IOP decrease (Mikawa et al., 1997) was observed. The maximum hypotensive effect occurred 1 or 2 hr after topical administration.

Subcutaneous indomethacin prevented the IOP effects of AEA (Pate et al., 1996). Indomethacin is a cyclo-oxygenase (COX) inhibitor, and prevents prostaglandin synthesis from arachidonic acid (Fig. 8). Earlier it had been suggested that it is a prostaglandin rather than arachidonic acid itself that affects IOP after arachidonic acid treatment (Podos et al., 1973a, 1973b). Thus, it seems that topically administered AEA is catabolized (Fig. 8) in the eye to arachidonic acid (see Section 4), which is subsequently biosynthesized to prostaglandins responsible for the IOP effects. Subcutaneous pretreatment with the FAAH inhibitor PMSF eliminated the typical initial increase of IOP observed in AEA-treated eyes, although a significant hypotension was apparent (Laine et al., 2002). When the experiment was repeated with a subcutaneous co-injection of SR 141716A (a highly specific CB_1 receptor antagonist), this hypotensive effect was eliminated, suggesting that IOP reduction caused by the apparently undergraded AEA may be mediated via a CB_1 receptor.

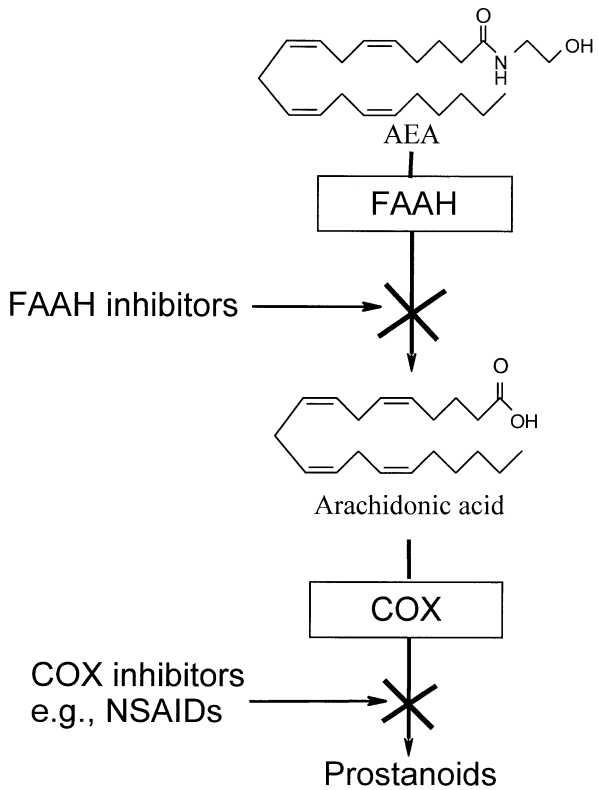

Fig. 8. Catabolism of AEA to arachidonic acid and subsequent biosynthesis of prostanoids.

5.4.2. α-Substituted anandamides

Topically administered α-substituted anandamides, such as α-methyl-AEA (Fig. 7) and α-isopropyl-AEA, lacked an initial increase of IOP, but caused immediate ocular hypotension in the treated eye (Pate et al., 1997). In the untreated eye, a significant IOP decrease was usually not observed. Subcutaneous injection (2.5 mg/kg) of SR 141716A eliminated the IOP reduction caused by topically administered α-isopropyl-AEA (Pate et al., 1998), which suggests that these IOP effects are mediated via a CB_1 receptor. Subcutaneous administration of SR 141716A alone elevated the IOP of rabbits, which may indicate that it is acting either as a competitive antagonist of endogenous AEA or as an inverse CB_1 receptor agonist (Bouaboula et al., 1997). In contrast, a topical AEA positive control, apparently working via a prostanoid mechanism, was unaffected by subcutaneously pre-administered SR 141716A, which indicates that the prevention of α-isopropyl-AEA effects by SR 141716A probably was not due to the sum of unrelated effects.

5.4.3. Anandamide uptake inhibitors

An interesting approach by Laine et al. (2001) for lowering IOP via a putative CB_1 mechanism was inferred from the works of Beltramo et al. (1997), Calignano et al. (1997), and Di Marzo et al. (1998a), and employed endocannabinoid transport inhibitors (Fig. 7) to increase levels of endogenous ocular AEA or 2-AG. They reported that a topical dose of AM404 (62.5 μg) administered in HP-β-CD significantly decreased IOP in rabbits. However, if AM404 was given in propylene glycol, a significant IOP increase (without subsequent IOP decrease) was observed. The authors concluded that the latter IOP increase may be due to a greater absorption of AM404 by the eye, followed by its degradation to arachidonic acid. In contrast, topical administration of olvanil (312.5 μg) in propylene glycol caused a significant IOP reduction, without provoking an initial hypertensive phase. This was thought most probably due to the fact that olvanil is a derivative of oleic acid (Fig. 7), which does not serve as a substrate for prostaglandin synthesis.

AM404 and olvanil activates vanilloid receptor Type 1 (VR1) (De Petrocellis et al., 2000). Consequently, it can be argued that the IOP effects of AM404 and olvanil might be mediated via VR1 receptors. However, it is not currently known if VR1 receptors are present in the eye, and if so, whether or not they are involved in regulating IOP. The IOP effects of selective endocannabinoid uptake inhibitors, such

as VDM11 and VDM13 (De Petrocellis et al., 2000), would be useful to study in order to eliminate the possible IOP effects via VR1 receptors.

5.5. Bimatoprost

Bimatoprost (Fig. 7) is a highly efficacious and long-acting ocular hypotensive agent (Woodward et al., 2001). It mimics the activity of a newly discovered family of fatty acid amides, termed "prostamides," that may be biosynthesized from AEA. Bimatoprost exhibits no activity on CB_1 or CB_2 receptors, which suggests that its IOP effects cannot be ascribed to an interaction with or stimulation of cannabinoid receptors. Bimatoprost is not classified as a cannabinoid due to its prostanoid chemical structure and lack of CB receptor activity.

5.6. HU-211

HU-211 (Fig. 5) is a non-psychoactive synthetic cannabinoid, although its enantiomer (HU-210) is one of the most potent cannabinoids described thus far (Devane et al., 1992a). HU-211 does not appreciably bind to the CB_1 receptor (Howlett et al., 1990), which explains this lack of cannabimimetic activity (Mechoulam et al., 1988). HU-211 is currently under evaluation as a commercial drug candidate for preventing the secondary biochemical injury that is collateral to head trauma or brain inflammation, probably via a *N*-methyl-D-aspartate (NMDA) receptor mechanism (Feigenbaum et al., 1989; Love, 1999).

Intravenous administration of HU-211 results in a dose-related IOP decrease in rabbits that is stronger than that of Δ^9-THC or Δ^8-THC (Beilin et al., 2000). This HU-211 effect can be attenuated by pretreatment with yohimbine (an α_2-adrenergic antagonist) and propranolol (a β-adrenergic antagonist).

A topical dose of HU-211 (0.12% as a submicron emulsion) significantly decreased IOP in normotensive rabbits (Naveh et al., 2000). The maximal IOP reduction was 5.3 ± 1.8 mm Hg at 1.5 hr after drug administration, and a statistically significant IOP decrease was also observed at 4 and 6 hr after drug administration. In the contralateral eye, a statistically significant IOP decrease was observed only at 4 hr after drug administration.

6. Mechanisms of action

6.1. Intraocular pressure reduction

The actual mechanism of action for cannabinoid reduction of IOP is unknown. Because smoking marijuana reduces blood pressure (Crawford & Merritt, 1979; Merritt et al., 1980a), the obvious hypothesis is that IOP reduction is simply a reflection of this change. Although systemic hypotension may have such an ocular influence, it probably cannot account for most of the observed IOP effect (Korczyn, 1980).

Until recently, the influence of ophthalmic cannabinoids on IOP has been assumed to be mediated through the CNS rather than locally. However, a substantial difference in IOP between the cannabinoid treated versus untreated eyes of cats (Colasanti et al., 1984a, 1984b, 1984c) supports the local effect hypothesis. Systemic absorption of the vast majority of a topically applied dose (Chiang et al., 1983) via blood circulation (Chang & Lee, 1987; Urtti & Salminen, 1993) and subsequent transfer of the drug to the untreated eye (Salminen & Urtti, 1984) may account for minor contralateral effects. Although the concentration of a drug in the untreated eye may be substantially lower than that of the treated eye, these lower concentrations are often sufficient to cause some reduction of IOP (Urtti & Salminen, 1985). This bilateral IOP asymmetry might also be explained as a combination of major localized and minor CNS effects. However, direct administration of THC into the cerebral ventricles of rabbits, or ventriculocisternal perfusion, does not affect IOP (Liu & Dacus, 1987).

Recent studies using the CB_1 receptor antagonist SR141716A have implied that the IOP reduction caused by cannabinoids is mediated via CB_1 receptors. Topically applied AEA (Pate et al., 1995) and other α-unsubstituted anandamides (Pate et al., 1996) seem to influence IOP through their hydrolysis to arachidonic acid, which is a COX pathway precursor of the prostanoids. In contrast, metabolically stable α-substituted anandamides (Pate et al., 1997) and other types of cannabinoids, e.g., CP-55,940 (Pate et al., 1998) and WIN-55,212-2 (Song & Slowey, 2000), seem to act via CB_1 receptors. However, simultaneous administration of the FAAH inhibitor PMSF apparently prevents degradation of exogenous AEA (Laine et al., 2002), as reflected by a disappearance of the typical initial hypertension. This apparently intact AEA acts upon a CB_1 receptor, as evidenced by the fact that its hypotensive effects could be eliminated via the use of SR141716A. An overall scheme for IOP physiology, including AEA metabolism, theorized from the studies published to date is presented in Fig. 9.

The anatomical distribution of ocular cannabinoid receptors (Straiker et al., 1999a) indicates that endogenous cannabinoids may have a physiological role for the regulation of ocular pressure. The existence of CB_1 receptors in the trabecular meshwork and in the Canal of Schlemm suggests a possible influence of cannabinoids on conventional aqueous humor outflow. CB_1 receptors of the ciliary pigment epithelium and ciliary muscle imply an effect on either (or both) aqueous humor production and uveoscleral outflow. Additional mechanistic studies are required to determine possible involvement of the sympathetic or parasympathetic nervous systems, and any possible vascular component (Kaufman & Wis, 1998).

The non-psychoactive synthetic cannabinoid HU-211 apparently reduces IOP (Naveh et al., 2000; Beilin et al.,

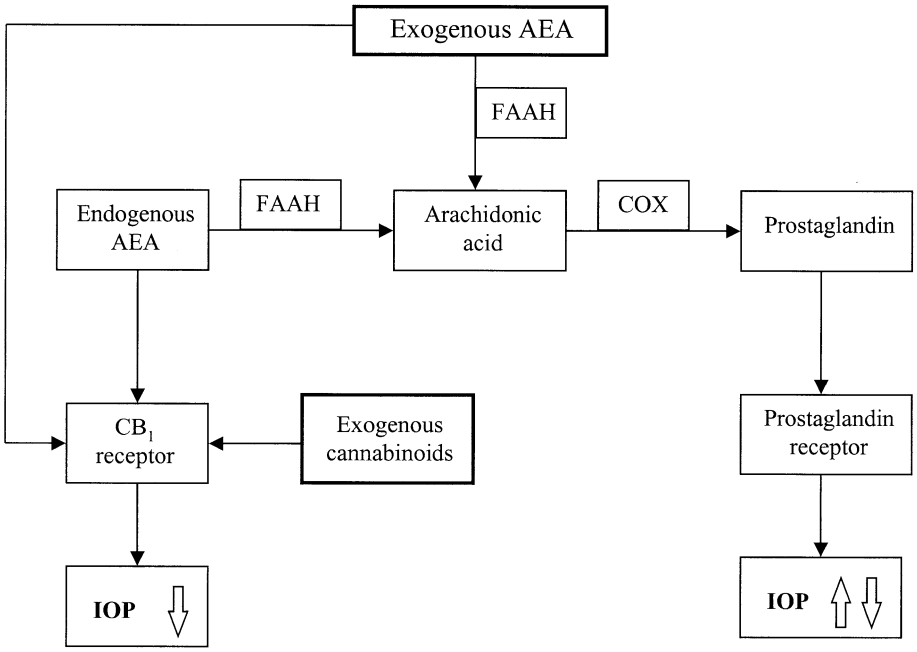

Fig. 9. The proposed ocular AEA metabolism and IOP dynamics.

2000), despite its relative inactivity at the CB_1 receptor (Howlett et al., 1990). Its mechanism of action is unknown at present. An attempt to counter this effect by the use of a CB receptor antagonist has not been attempted yet. HU-211 is an NMDA receptor antagonist (Feigenbaum et al., 1989; Nadler et al., 1993), and to our knowledge, evidence correlating NMDA receptor antagonism to a decrease in IOP has yet to be published.

6.2. Neurological implications

Elevated IOP is only one factor in the pathophysiology of glaucoma, and its influence on the retinal disk is indirect. Indeed, a significant percentage of glaucoma patients exhibit normotensive IOP (Sommer et al., 1991; Collaborative Normal Tension Glaucoma Study Group, 1998a, 1998b). At present, it is commonly accepted that glaucoma is a degenerative disease of the optic nerve (Schwartz & Yoles, 2000). Retinal disc damage is thought to occur through at least two mechanisms: occlusion of axonal flow, which causes localized interference with delivery of organelles (e.g., Hochmann & Herkenham, 1999) and cytosolic factors (Nickells, 1999), and restriction of the microcirculation that nourishes the optic nerve (Prunte et al., 1998). While both phenomena are influenced by IOP, the latter may also occur independently.

On the intracellular level, retinal disk ischemia causes anoxia and hypoglycemia, resulting in neuronal toxicity and ultimately in apoptosis (Nickells, 1999; Nickells & Zack, 1996). This, in turn, contributes to a toxic extracellular environment that may result in an ongoing progression of optic nerve degeneration (Schwartz & Yoles, 1999). The secondary neuronal injury observed in "stroke" victims acts by a similar mechanism (Love, 1999). Among the products produced by this process are an excess of neurotransmitters, such as glutamate (Duarte et al., 1998; Sucher et al., 1997) and nitric oxide (Adachi et al., 1998a, 1998b). The former compound induces a neuronal "hyperexcitability" of the NMDA receptor and results in a large flux of Ca^{2+} (Pauwels et al., 1991; Ritch, 2000; Schousboe et al., 1997) and other ions (Pauwels et al., 1991; Yu et al., 1999) across the membrane, which induces cell death. Excessive quantities of nitric oxide synergize apoptosis (Leist et al., 1997) and act as an oxidative free radical initiator, which induces a cascade of other destructive effects, including lipid membrane peroxidation. Tumor necrosis factor-α has been suggested (Shohami et al., 1997) to be a primary mediator of neurotoxicity after brain trauma, and its possible involvement in retinal ischemia also bears scrutiny.

Although the effect of HU-211 on vascular tissues is unknown, it shares many of the neurotherapeutic effects of THC. It has been investigated for use in both secondary brain (Leker et al., 1999) and optic nerve (Yoles et al., 1996; Schwartz & Yoles, 1999) injury. Its actions include a combination of effects: open channel blockade of the NMDA Ca^{2+} channel (Eshhar et al., 1993; Nadler et al., 1993), non-glutamate/glycine receptor antagonism (Feigenbaum et al., 1989), tumor necrosis factor-α inhibition (Shohami et al., 1997), nitric oxide suppression (Gallily et al., 1997), and free radical scavenging (Biegon & Joseph, 1995). Ca^{2+}-channel blockers have shown promise for clinical use in glaucoma (Netland et al., 1993), and the ability of HU-211 to also antagonize NMDA receptor activity is complementary. Interestingly, AEA has demonstrated an ability to modulate this receptor (Hampson et al.,

1998b), and palmitoylethanolamide has also been shown to exhibit an indirect anti-excitotoxic effect involving a CB_2-like receptor (Skaper et al., 1996).

The overall implication for the sum of these studies is potentially significant. Cannabinoids may reveal themselves to be useful for the treatment of glaucoma in a quite comprehensive manner: lowering IOP, restoring microcirculation, inhibiting apoptosis, and minimizing free radical damage, among other mechanisms. This hypothetical combination would supersede that employed by any currently employed glaucoma drug, and may help to explain why the smoking of marijuana has preserved the sight of those unresponsive to other glaucoma therapies.

7. Cannabinoids for glaucoma therapy

It is well documented that various cannabinoids are able to reduce IOP when administered orally, intravenously, or by inhalation (e.g., Green, 1998). Generally, it is thought that the clinical use of cannabinoids in the treatment of glaucoma is hindered by difficulties with preparing appropriate ophthalmic dosage forms and because of their potential for psychoactive side-effects. Novel drug delivery technologies, such as CDs (Jarho et al., 1996b, 1998) or microemulsions (Muchtar et al., 1992), have enabled preparation of topically administered cannabinoids that decrease IOP. This is a clear improvement over lipid-based vehicles such as the oils used in earlier studies of topical cannabinoids. The question of CNS side-effects is a more open question, although moot if the total delivered dose is less than that needed to elicit psychoactivity. The following approaches may provide the means by which an ophthalmic cannabinoid pharmaceutical can be developed.

7.1. Optimal drug delivery properties

Only a few percent of an ophthalmic dose is delivered to the intraocular tissues. The major part of this dose will be absorbed into the systemic circulation, which often leads to side-effects (Fig. 1). If ocular absorption can be improved by formulation approaches or by molecular modification, the therapeutic goal can be achieved by a smaller topical dose, with consequent reduction of undesired effects. This is an especially important consideration in the development of ophthalmic medicines affecting CB_1 receptors. The pro-drug strategy may also improve the physicochemical and biopharmaceutical properties of ophthalmic cannabinoids (Järvinen & Järvinen, 1996), and has been applied successfully to the development of other glaucoma drugs, e.g., dipivefrin (Mandell et al., 1978) and latanoprost (Resul et al., 1993). Recently, O-1057 was reported as a possible water-soluble prodrug of THC derivation (Pertwee et al., 2000) and anandamide phosphates as potential water-soluble prodrugs of anandamides (Järvinen et al., 2001).

7.2. CB_2 receptor approach

Recent IOP (Pate et al., 1998; Laine et al., 2002; Song & Slowey, 2000) and receptor (Straiker et al., 1999a, 1999b; Yazulla et al., 1999, 2000) studies strongly suggest that IOP effects of cannabinoids are mediated via ocular CB_1 receptors. However, studies should be carried out to determine if populations of CB_2 receptors exist on anatomically relevant areas of the eye and if CB_2-specific receptor agonists or antagonists have an IOP effect.

7.3. Cannabinoid "soft-drugs"

A "soft-drug" is designed to undergo rapid metabolic deactivation after affecting its target area. An example of ophthalmic application of this drug strategy is loteprednol etabonate, an active corticosteroid that lacks the usual steroidal systemic side-effects (Bodor, 2000). An ideal "soft" ophthalmic cannabinoid would be absorbed by the eye and have a pharmacological effect, but would become inactivated in the systemic circulation, thus limiting its effect on target tissues. Several soft cannabinoids are currently under development (Buchwald et al., 2000).

After topical administration, AEA could potentially act as an ophthalmic soft-drug to decrease IOP via an ocular CB_1 receptor. However the catabolism of its amide bond leading to the formation of CB_1-inactive arachidonic acid is actually too rapid and results in an elevation of IOP. Adding an α-carbon substituent protects against this metabolic vulnerability, defaulting the molecule to a slower metabolism at other structural locations (e.g., double bonds) and allowing CB_1 activity in the eye.

8. Conclusions

Rapid advances in cannabinoid research have been achieved in less than a decade. These characterizations have provided basic tools for a closer look at the mechanisms behind the well-known efficacy of marijuana on glaucoma. The recent demonstration of endocannabinoid IOP mechanisms and the subsequent discovery of CB_1 receptors on appropriate intraocular structures suggests a physiological role for these compounds in the regulation of normal ocular tension, and implies a route by which a new class of anandamide-based glaucoma drugs may be developed. Topical delivery of these or other cannabinoids directly to the site of action should eliminate psychoactivity, but suffer practical problems of administration, which can be overcome through modern techniques of molecular design and drug delivery. The prospect of cannabinoid glaucoma medicines promises someday to substitute simple IOP reduction for a more comprehensive package of ocular therapeutic support.

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