

## SHORT COMMUNICATION

# The synthetic cannabinoid WIN55212-2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies

Anna Porcella, Chiara Maxia,<sup>1</sup> Gian Luigi Gessa<sup>2</sup> and Luca Pani<sup>2</sup>

Center for Neuropharmacology C.N.R., B.B. Brodie Department of Neuroscience, University of Cagliari, via Porcell 4, 09124-I Cagliari, Italy

<sup>1</sup>Ophthalmology Division A.S.L. 8, Ospedale S. Michele, via Peretti 12. 09124-I Cagliari, Italy

<sup>2</sup>Neuroscienze Scarl, Cagliari, Italy

**Keywords:** CB<sub>1</sub>, ciliary body, eye, marijuana

### Abstract

The search for new ocular hypotensive agents represents a frontier of current eye research because blindness due to optic neuropathy occurs insidiously in 10% of all patients affected by glaucoma. Cannabinoids have been proposed to lower intraocular pressure by either central or peripheral effects but a specific mechanism for this action has never been elucidated. We recently demonstrated the presence of the central cannabinoid receptor (CB<sub>1</sub>) mRNA and protein in the human ciliary body. In the present study we show that the synthetic CB<sub>1</sub> receptor agonist, WIN 55212–2, applied topically at doses of 25 or 50 µg ( $n = 8$ ), decreases the intraocular pressure of human glaucoma resistant to conventional therapies within the first 30 min ( $15 \pm 0.5\%$  and  $23 \pm 0.9\%$ , respectively). A maximal reduction of  $20 \pm 0.7\%$  and  $31 \pm 0.6\%$ , respectively, is reached in the first 60 min. These data confirm that CB<sub>1</sub> receptors have direct involvement in the regulation of human intraocular pressure, and suggest that, among various classes of promising antiglaucoma agents, synthetic CB<sub>1</sub> receptor agonists should deserve further research and clinical development.

### Introduction

Glaucoma is mostly a painless, insidious form of optic neuropathy which leads to vision loss. It is estimated that almost seven million people worldwide are totally blind, owing to the progression of their glaucoma. This represents about 1/10th of all people affected by this disease (Quigley, 1996). As glaucoma is a chronic disease lacking a cure, the quest for new ocular hypotensive agents is important for its treatment, and these agents are likely to remain frontline therapy for the foreseeable future (Sugrue, 1997).

Current pharmacological therapies are based on lowering intraocular pressure (IOP), one of the risk factors that can be decreased in open-angle glaucoma, the most common of all forms of glaucoma (Alward, 1998). Most drugs for glaucoma are applied topically and are well tolerated. However, severe systemic side effects can occur, up to the point where the side effects should always be considered when a patient, undergoing local ocular therapy, presents with new systemic problems (Sugrue, 1997). Drugs currently employed for long-term glaucoma therapy belong to five groups: (i) beta-adrenergic antagonists; (ii) cholinergic agonists; (iii) carbonic anhydrase inhibitors; (iv) alpha-2 adrenergic agonists and (v) prostaglandin

analogues, all of which may have important general, as well as specific, central nervous system, cardiovascular, gastrointestinal, pulmonary and haematologic systemic side effects (McLaughlin & Chiou, 1985; Alward, 1998).

Since the early 1970s it was reported that smoking marijuana cigarettes could lower IOP by up to 45% (Hepler & Frank, 1971); later works showed that the principal active ingredient in marijuana, Delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC), lowered IOP when given intravenously (Purnell & Gregg, 1975), orally (Merritt et al., 1980a) or by inhalation (Merritt et al., 1980b). Others have failed to demonstrate any efficacy of cannabinoid compounds in lowering IOP (Pate et al., 1996; Hodges et al., 1997), concluding that side effects such as hypotension, tachycardia, palpitations and altered mental status precluded the use of these drugs in glaucomatous patients. Cannabinol, nabilone,  $\Delta^9$ -THC and  $\Delta^8$ -THC have been found to decrease IOP in humans affected with glaucoma, whereas cannabidiol had no effect (Elsohly et al., 1981, 1984).

We recently found that rat (Porcella et al., 1998) as well as human (Porcella et al. 2000) eyes are rich in CB<sub>1</sub> mRNA and protein, supporting a direct role for the CB<sub>1</sub> receptor in controlling IOP. The significance of this finding has been confirmed in rabbits by the IOP lowering effect of the aminoalkylindole, WIN55212-2, a synthetic and selective CB<sub>1</sub> receptor agonist (Song & Slowey, 2000). The present study was undertaken in order to determine whether WIN55212-2 would also show any effect on the IOP of human glaucoma resistant to conventional therapies.

**Correspondence:** Dr Luca Pani, Center for Neuropharmacology C.N.R., B.B. Brodie Department of Neuroscience, University of Cagliari, via Porcell 4, 09124-I Cagliari, Italy  
E.mail: panil@unica.it

Received 17 August 2000, revised 14 November 2000, accepted 16 November 2000

TABLE 1. Patient demographic, diagnostic and therapeutic data

Patient no.	Patient	Sex	Age (years)	Glaucoma type	IOP	Therapy
1	GM	F	72	Closed angle	22	Acetaz + physostigmine
2	RP	F	27	Familial malformative	25	$\beta$ -blocker + pilocarpine
3	FP	M	21	Familial malformative	31	$\beta$ -blocker + pilocarpine
4	EM	F	53	Pigmentary with high myopia	26	$\beta$ -blocker + pilocarpine + physostigmine
5	PC	M	35	Open angle	22	$\beta$ -blocker + pilocarpine
6	EDC	M	53	Simple primary chronic	23	$\beta$ -blocker + pilocarpine
7	SC	M	63	Simple primary chronic	22	$\beta$ -blocker + pilocarpine
8	AS	M	60	Simple primary chronic	24	$\beta$ -blocker + pilocarpine + physostigmine

Acetaz, acetazolamide;  $\beta$ -blocker, thymolol.

## Patients and methods

### Patient population

Eight patients attending the glaucoma service of S. Michele Hospital, Cagliari, Italy and under the care of one board-certified ophthalmologist (C.M.), were enrolled in the study (Table 1). Patients with bilateral glaucoma being treated with multiple topical medical therapy, were selected. One eye was randomly chosen to be treated by an investigator who was unaware of the primary diagnosis or the IOP values. In all cases, the indication for synthetic cannabinoid was inadequate control of IOP (i.e. values >22 mmHg) with their current therapy. Although the group consisted of patients who had differing ophthalmic histories, each patient acted as his/her own control. The protocol and procedures of this work were approved by a local Ethical Committee. Informed written consent was obtained from all patients according to the Declaration of Helsinki World Medical Assembly in Helsinki (1964).

On admittance to the study, a standard protocol was used for all patients. A general check-up and an initial ocular evaluation was performed which included Snellen visual acuity, Goldmann applanation tonometry, examination of the ocular adnexa, slit-lamp biomicroscopy, gonioscopy and a dilated stereoscopic evaluation of the fundus using the Volk 90-diopter lens. A perimeter [Octopus 500 EZ, G1 program; analysis by Peridata 6.2c (Interzeag AG, Zurich, Switzerland)] was used for testing the visual field. Demographic, diagnostic and therapeutic data are shown in Table 1. All patient were in wash-out from their previous therapy for 12 h.

### Preparation of drug and eyedrops

The synthetic cannabinoid receptor agonist WIN55212-2 was purchased from Tocris Cookson Ltd. (Bristol, United Kingdom). The low aqueous solubility of WIN55212-2 was overcome through the use of 45% 2-hydroxypropyl- $\beta$ -cyclodextrin (Sigma-Aldrich, Milan, Italy). A 150- $\mu$ g stock solution was made by dissolving WIN55212-2 in 500  $\mu$ L of 150 mM NaCl, and then diluted with sterile saline. The vehicle control solution contained 150 mM NaCl and 45% 2-hydroxypropyl- $\beta$ -cyclodextrin. This vehicle has been successfully used to deliver anandamide to the rabbit eye, and is neither irritant nor toxic to the eye (Song & Slowey, 2000). The pH of these solutions was adjusted to 7.4 with NaOH, and they were filter-sterilized through 0.2- $\mu$ m pore disposable filters (Waters, Milan, Italy). Before employing such preparations on the patients, the stock solution was tested on a volunteer (L.P.) to assure that it was neither an irritant nor that it could produce any major side effects.

### Measurement of IOP

Before the measurement of IOP, 25  $\mu$ L of 0.05% tetracaine solution was applied topically to the eye for local anaesthesia. All subsequent

IOP readings were taken after local anaesthesia; tetracaine did not affect IOP significantly. To perform each evaluation, two drops (50  $\mu$ L) of a 25 or 50  $\mu$ g WIN55212-2 or vehicle solution were instilled unilaterally on the lower corneoscleral limbus. During instillation the lower eyelid was slightly pulled away from the globe, and gentle finger pressure was applied to the nasolacrimal channel for 1 min. IOP was measured using the Goldman applanation tonometer, always by the same observer (who was unaware of which eye was being treated). For every determination at least two readings were taken from each treated (ipsilateral) and untreated (controlateral) eye, and the mean of these readings was used. The IOP of the patients was measured from 30 min before to 3 h after eye drop administration, at 15 min intervals. IOP at the time of eye drop administration (0 h) was considered as the baseline value. Baseline values ranged between 22 and 31 mmHg. To avoid significant fluctuation in the diurnal curve of the IOP, which may be an important factor in predicting any subsequent poor response to the topical therapy (Konstas *et al.*, 1997), the evaluation sessions were repeated twice with an interval of one week, once in the morning (9 a.m.) and once in the late afternoon (6 p.m.). No significant differences were observed in the two measurement sessions.

### Analysis of data and statistics

The data presented in the Fig. 1 represent mean  $\pm$  SE values. The data were analysed using Prism (Graph PAD Software, San Diego, CA, USA) and plotted as IOP values (mmHg) vs time (h). The statistical significance of the effect of WIN55212-2 on the IOP values of treated eye was evaluated by one-way analysis of variance (ANOVA). When a significant ( $P < 0.05$ ) interaction (vehicle vs drug treatment) was demonstrated, the Newman-Keuls *post hoc* test was used to compare the effect of the instilled drug from that of the vehicle.

## Results

### IOP-lowering effect of WIN55212-2

Figure 1 shows the time course and dose-response relationship for the topical administration of WIN55212-2 on IOP. WIN55212-2 promptly decreased IOP in most patients treated, and a dose response was evident in the first 30 min by comparing the 25  $\mu$ g ( $-15 \pm 0.5\%$ ) with the 50  $\mu$ g ( $-23 \pm 0.9\%$ ) dose. In both series of patients the maximal effect was observed after 60 min from the instillation of the drug where the decreased percentage with the two dosages used was equal to  $20 \pm 0.7$  and  $31 \pm 0.6\%$ , respectively. Interestingly, at this time point an effect was also observed in the untreated eye, although it never reached statistical significance with respect to baseline values.

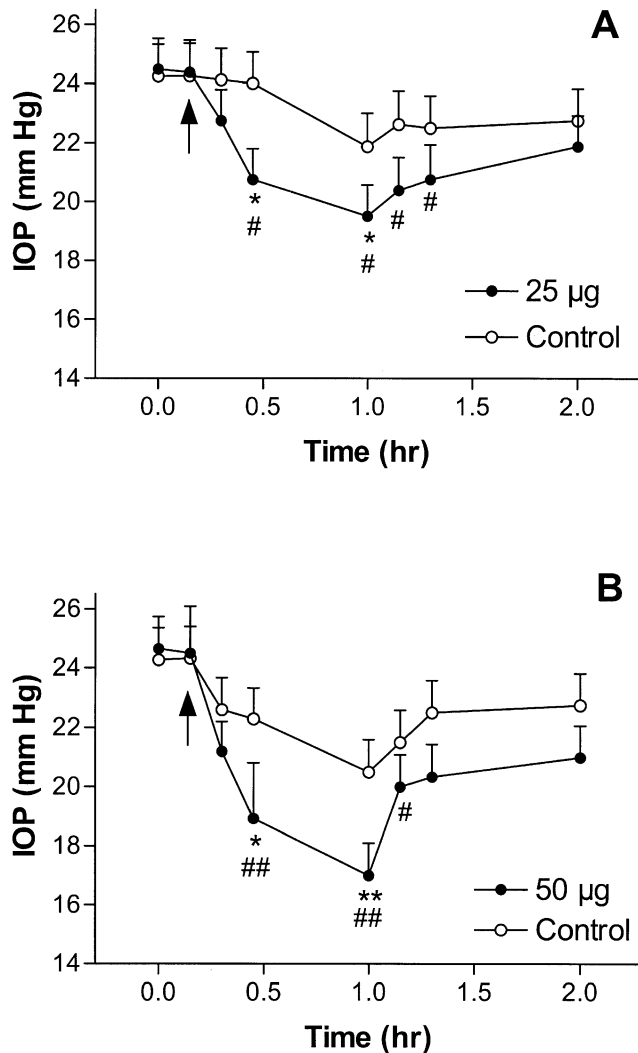


FIG. 1. Effect of topical administration of WIN55212-2 at two different doses, 25 (panel A) and 50 µg (panel B), on intraocular pressure of glaucomatous patients ( $n = 8$ ) resistant to conventional therapies. # $P < 0.05$  and ## $P < 0.01$  with respect to baseline values (both eyes). \* $P < 0.05$  and \*\* $P < 0.01$  with respect to untreated eye.

## Discussion

Hepler & Frank (1971) showed that marijuana smoking could significantly decrease IOP in volunteers and later research showed that this effect could be attributable to  $\Delta^9$ -THC when given to healthy individuals (Purnell & Gregg, 1975) or to glaucomatous patients (Merritt et al., 1980a), ruling out a simple IOP stabilizing effect of cannabinoids (Mechoulam et al., 1998; Pertwee, 1999).

The role of CB<sub>1</sub> receptors in decreasing IOP in normotensive rabbits, after the topical administration of the endogenous ligand arachidonyl ethanolamide (Pate et al., 1996), was brought into question by the lack of action of systemically administered WIN55212-2 (Hodges et al., 1997). However, very recently, it was shown that, in rabbits, the topical administration of WIN55212-2 was able to lower IOP in a time and dose dependent manner (Song & Slowey, 2000). In the current study, we observed that in glaucomatous patients, the IOP-lowering effects of WIN55212-2 were time and dose dependent, with no significant effect in the contralateral eye, indicating that the effects of WIN55212-2 were not

due to systemic actions but rather mediated by cannabinoid receptors in the eye.

This confirms our and other's recent findings in the rat (Porcella et al., 1998) and human (Straiker et al., 1999; Porcella et al. 2000) ciliary bodies of significantly detectable levels of CB<sub>1</sub> transcripts and proteins. CB<sub>1</sub> cannabinoid receptors were also found expressed in the embryonic rat retina suggesting that cannabinoids could influence eye development (Buckley et al., 1998) and disrupt habituation and reactivity to different illumination conditions in adulthood (Navarro et al., 1995). Accordingly, in humans, prenatal exposure to marijuana was found to decrease visual perception (Fried, 1995).

The mechanisms by which cannabinoids are able to decrease IOP remain open to debate. Indirect mechanisms of action have been proposed as being responsible for the CB<sub>1</sub> receptor mediated IOP-lowering effect in the human eye. Inhibition of presynaptic calcium channels (Twitchell et al., 1997) by cannabinoids may reduce noradrenaline release in ocular tissues, in a similar way to what has been demonstrated in slices from the human and rat hippocampus (Schlicker et al., 1997), and decrease the production of aqueous humor that depends on the adrenergic tone produced by  $\alpha_2$  and  $\beta$  receptors localized in the ciliary body (Sugrue, 1997). In contrast to this hypothesis, both  $\alpha$ - and  $\beta$ -adrenergic antagonists, however, reduce the  $\Delta^9$ -THC-induced decrease in IOP by approximately 50% (Green & Kim, 1976). It could be possible that, rather than reducing the production of aqueous humor, cannabinoids may favour the opening of the endothelial Schlemm-Fontana channels that drain the aqueous humor. The dimensions of Schlemm's canal in glaucomatous human eyes have been found significantly smaller than those in normal eyes. (Allingham et al., 1996).

The high level of CB<sub>1</sub> mRNA and functional protein found in the human ciliary body (Porcella et al. 2000) supports the hypothesis that cannabinoids may act directly as vasodilators of the efferent blood vessels of the anterior uvea, favouring humor aqueous efflux. This effect on the endothelial eye structure is reminiscent of the improved microcirculation due to a localized vasodilation (Randall & Kendall, 1998) of vascular smooth cells challenged with macrophage-derived anandamide (Wagner et al., 1997).

In addition, in laboratory animals, the IOP-lowering effect of CP-55940 (Pate et al., 1998) and WIN55212-2 (Song & Slowey, 2000), two structurally distinct compounds with comparative potency consistent with their affinity for cannabinoid receptors (Compton et al., 1996), were shown to both be eliminated by the specific cannabinoid receptor antagonist SR141716A, suggesting that their effect is most likely mediated through cannabinoid receptors expressed in the eye. In addition, the finding that SR141716A *per se*, whether given systemically (Pate et al., 1998) or applied locally (Song & Slowey, 2000) increases IOP, is consistent with an inverse agonism (Bouaboula et al., 1997) or an antagonism by SR141716A of endogenous cannabinoid tone on IOP.

Anandamide and congeners formation in the nervous system has been shown to occur, by multiple biochemical pathways, through phosphodiesterase-mediated cleavage of a phospholipid precursor, N-arachidonoyl-phosphatidylethanolamine (Di et al., 1994). Enzyme activity for anandamide synthesis has been reported in porcine ocular tissue (Matsuda et al., 1997) and locally produced anandamide, either in the ocular tissue (Matsuda et al., 1997) or macrophage (Wagner et al., 1997), could be responsible for the vasodilatation mediated by a CB<sub>1</sub> receptors.

Since ethical reasons advised against the use of a CB<sub>1</sub> antagonist to be employed in the present study, we cannot be entirely certain that the observed IOP-lowering effect of WIN55212-2 is mediated by a CB<sub>1</sub> receptor. It is also conceivable that other known or unknown

cannabinoid receptor subtypes or nonreceptor related mechanisms may be involved.

In conclusion, our results suggest that the topical application of WIN55212-2 lowers IOP in human glaucoma, and that the IOP-lowering effects of WIN55212-2 are, most likely, directly mediated through a CB<sub>1</sub> cannabinoid receptor. These present data would encourage further clinical research and development of CB<sub>1</sub> receptor agonists as potential antiglaucoma agents.

## Acknowledgements

This work was supported by a grant from the National Research Council and by Regione Autonoma Sardegna.

## Abbreviations

CB<sub>1</sub>, central cannabinoid receptor subtype 1; IOP, intraocular pressure; Δ<sup>9</sup>-THC, delta-9-tetrahydrocannabinol; Δ<sup>8</sup>-THC, delta-8-tetrahydrocannabinol.

## References

- Allingham, R.R., de Kater, A.W. & Ethier, C.R. (1996) Schlemm's canal and primary open angle glaucoma: correlation between Schlemm's canal dimensions and outflow facility. *Exp. Eye Res.*, **62**, 101–109.
- Alward, W.L.M. (1998) Medical management of Glaucoma. *N. Engl. J. Med.*, **339**, 1298–1307.
- Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.P., Le Fur, G. & Casellas, P. (1997) A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J. Biol. Chem.*, **35**, 22330–22339.
- Buckley, N.E., Hansson, S., Harta, G. & Mezey, E. (1998) Expression of the CB<sub>1</sub> and CB<sub>2</sub> receptor messenger RNAs during embryonic development in the rat. *Neuroscience*, **82**, 1131–1149.
- Compton, D.R., Harris, L.S., Litchman, A.H. & Martin, B.R. (1996) Marihuana. In Schuster, C.R. & Kuhar, M.J. (eds), *Handbook of Experimental Pharmacology: Pharmacological Aspects of Drug Dependence*. Springer, Berlin, p. 83.
- World Medical Assembly in Helsinki (1984) Declaration of Helsinki Adopted in 1964 by the 18th World Medical Assembly in Helsinki, Finland, and revised by the 29th World Medical Assembly in Tokyo in 1975. In Andrew C. Varga (ed), *The Main Issue in Bioethics* (Revised Edn). Paulist Press, New York. pp. 45–50.
- Di, M.V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C. & Piomelli, D. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*, **372**, 686–691.
- Elsohly, M.A., Harland, E.C., Benigni, D.A. & Waller, C.W. (1984) Cannabinoids in glaucoma II: the effect of different cannabinoids on intraocular pressure of the rabbit. *Curr. Eye Res.*, **3**, 841–850.
- Elsohly, M.A., Harland, E.C., Murphy, J.C., Wirth, P. & Waller, C.W. (1981) Cannabinoids in glaucoma: a primary screening procedure. *J. Clin. Pharmacol.*, **21**, 472S–478S.
- Fried, P.A. (1995) The Ottawa prenatal prospective study (OPPS): methodological issues and findings – it's easy to throw the baby out with the bath water. *Life Sci.*, **56**, 2159–2168.
- Green, K. & Kim, K. (1976) Interaction of adrenergic antagonists with prostaglandins E<sub>2</sub> and tetrahydrocannabinol in the eye. *Invest. Ophthalmol.*, **15**, 102–112.
- Heppler, R.S. & Frank, I.R. (1971) Marihuana smoking and intraocular pressure. *JAMA*, **217**, 1392.
- Hodges, L.C., Reggio, P.H. & Green, K. (1997) Evidence against cannabinoid receptor involvement in intraocular pressure effects of cannabinoids in rabbits. *Ophthalmic Res.*, **29**, 1–5.
- Konstas, A.G.P., Mantziris, D.A. & Stewart, W.C. (1997) Diurnal intraocular pressure in untreated exfoliation and primary open-angle glaucoma. *Arch. Ophthalmol.*, **115**, 182–185.
- Matsuda, S., Kanemitsu, N., Nakamura, A., Mimura, Y., Ueda, N., Kurahashi, Y. & Yamamoto, S. (1997) Metabolism of anandamide, an endogenous cannabinoid receptor ligand, in porcine ocular tissue. *Exp. Eye Res.*, **64**, 707–711.
- McLaughlin, M.A. & Chiou, G.C. (1985) A synopsis of recent developments in antiglaucoma drugs. *J. Ocul. Pharmacol.*, **1**, 101–121.
- Mechoulam, R., Hanus, L. & Fride, E. (1998) Towards cannabinoid drugs revisited. *Prog. Med. Chem.*, **35**, 199–243.
- Merritt, J.C. (1982) Glaucoma, hypertension, and marijuana. *J. Natl Med. Assoc.*, **74**, 715–716.
- Merritt, J.C., Crawford, W.J., Alexander, P.C., Anduze, A.L. & Gelbart, S.S. (1980b) Effect of marijuana on intraocular and blood pressure in glaucoma. *Ophthalmology*, **87**, 222–228.
- Merritt, J.C., McKinnon, S., Armstrong, J.R., Hatem, G. & Reid, L.A. (1980a) Oral Delta (9) -tetrahydrocannabinol, in heterogenous glaucoma. *Ann. Ophthalmol.*, **12**, 947–950.
- Navarro, M., Rubio, P. & Rodriguez de Fonseca, F. (1995) Behavioural consequences of maternal exposure to natural cannabinoids in rats. *Psychopharmacology*, **122**, 1–14.
- Pate, D.W., Järvinen, K., Urtili, A., Jarho, D., Fich, M., Mahadevan, V. & Järvinen, T. (1996) Effects of topical anandamides on intraocular pressure in normotensive rabbits. *Life Sci.*, **21**, 1849–1860.
- Pate, D.W., Jarvinen, K., Urtili, A., Mahadevan, V. & Jarvinen, T. (1998) Effect of the CB<sub>1</sub> receptor antagonist, SR141716A, on cannabinoid-induced ocular hypotension in normotensive rabbits. *Life Sci.*, **63**, 2181–2188.
- Pertwee, R.G. (1999) Cannabis and cannabinoids: pharmacology and rationale for clinical use. *Forsch. Komplementarmed.*, **6**, 12–15.
- Porcella, A., Casellas, P., Gessa, G.L. & Pani, L. (1998) Cannabinoid receptor CB<sub>1</sub> mRNA is highly expressed in the rat ciliary body: implications for the antiglaucoma properties of marihuana. *Brain Res.*, **58**, 240–245.
- Porcella, A., Maxia, C., Gessa, G.L. & Pani, L. (2000) The human eye expresses high levels of CB<sub>1</sub> cannabinoid receptor mRNA and protein. *Eur. J. Neurosci.*, **12**, 1123–1127.
- Purnell, W.D. & Gregg, J.M. (1975) Delta (9) -tetrahydrocannabinol, euphoria and intraocular pressure in man. *Ann. Ophthalmol.*, **7**, 921–923.
- Quigley, H.A. (1996) Number of people with glaucoma worldwide. *Br. J. Ophthalmol.*, **80**, 389–393.
- Randall, M.D. & Kendall, D.A. (1998) Endocannabinoids: a new class of vasoactive substances. *Trends Pharmacol. Sci.*, **19**, 55–58.
- Schlicker, E., Timm, J., Zentner, J. & Gothert, M. (1997) Cannabinoid CB<sub>1</sub> receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **356**, 583–589.
- Song, Z.H. & Slowey, C.A. (2000) Involvement of cannabinoid receptors in the intraocular pressure-lowering effects of WIN55212-2. *J. Pharmacol. Exp. Ther.*, **292**, 136–139.
- Straiker, A., Stella, N., Piomelli, D., Mackie, K., Karten, H.J., Maguire, G. (1999) Cannabinoid CB<sub>1</sub> receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system, *Proc. Natl Acad. Sci. USA*, **25**, 14565–14570.
- Sugrue, M.F. (1997) New Approaches to Antiglaucoma Therapy, *J. Med. Chem.*, **40**, 2793–2809.
- Twitchell, W., Brown, S. & Mackie, K. (1997) Cannabinoids inhibit N and P/Q-type calcium currents in cultured rat hippocampal neurons. *J. Neurophysiol.*, **78**, 43–50.
- Wagner, J.A., Varga, K., Ellis, E.F., Rzigalinski, B.A., Martin, B.R. & Kunos, G. (1997) Activation of peripheral CB<sub>1</sub> cannabinoid receptors in haemorrhagic shock. *Nature*, **390**, 518–521.