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Intraocular Pressure, Ocular Toxicity and Neurotoxicity after Administration of Δ^9-Tetrahydrocannabinol or Cannabichromene

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Δ-9-Tetrahydrocannabinol (Δ^9-THC) or cannabichromene, a structurally diverse naturally occurring cannabinoid, was delivered unilaterally to the corneas of cats either acutely by application of single drops or chronically via osmotic minipumps over a period of nine days. While Δ^9-THC only reduced intraocular pressure (IOP) minimally after acute administration, this cannabinoid produced substantial reductions in ocular tension during the entire period of chronic administration. Ocular toxicity during chronic treatment, however, was pronounced; conjunctival chemosis, erythema, and hyperemia were sustained, and corneal opacities approximating the site of drug delivery became evident within three to five days. In contrast, cannabichromene did not significantly alter IOP either acutely or during the nine days of chronic administration, and ocular toxicity was not apparent.

After systemic administration of Δ^9-THC to rats, a dose-related increase in the appearance of 8–13 Hz polyspike discharges became evident in the electrocorticogram during wakefulness and behavioral depression. These polyspikes subsequently reappeared during rapid eye movement (REM) sleep episodes. Cannabichromene was devoid of this effect.

These results indicate that, in contrast with acute administration, chronic delivery of Δ^9-THC to cat eyes produces substantial reductions in IOP. The tension lowering effect, however, is accompanied by considerable ocular toxicity and neurotoxicity. As cannabichromene lacked these activities, the terpenoid portion of the cannabinoid structure appears to be important for their mediation.

Key words: cannabinoids; Δ^9-tetrahydrocannabinol; cannabichromene; intraocular pressure; ocular toxicity; neurotoxicity; cats; rats.

1. Introduction

Since the initial observation of the IOP-lowering effect of marihuana by Hepler and Frank (1971), numerous investigations have been directed toward an understanding of this phenomenon. The primary psychoactive constituent of marihuana, Δ^9-THC, has been reported to lower IOP in rabbits (Green and Pederson, 1973; Green and Bowman, 1976; Green, Bigger, Kim and Bowman, 1977a), in dogs (Merritt et al., 1981b), in monkeys (Green and Kim, 1977), and in humans (Purnell and Gregg, 1975; Cooler and Gregg, 1976; Merritt, Olsen, Armstrong and McKinnon, 1981a). Δ-8-tetrahydrocannabinol, which is chemically closely related to Δ^9-THC and is also psychoactive, was likewise reported to produce a moderate IOP-lowering effect in rabbits (Green, Wynn and Bowman, 1978) and in man (Perez-Reyes, Wagner, Wall and Davis, 1976). Cannabinol is a naturally occurring cannabinoid which possesses only a moderate degree of psychoactivity (Perez-Reyes, Timmous, Davis and Wall, 1973). This cannabinoid likewise produced only a modest lowering of IOP in rabbits (Green et al., 1978). On the other hand, cannabidiol, a major marihuana cannabinoid which is devoid of psychic effects (Perez-Reyes et al., 1973; Hollister, 1974), was as effective in lowering IOP as Δ^9-THC in rabbits (Green et al., 1978). This cannabinoid,

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however, exerted only a minimal effect on ocular tension after intravenous administration to man (Perez-Reyes et al., 1976).

Several adverse ocular effects have been reported to accompany the fall in IOP induced by marihuana or Δ^9-THC. Conjunctival hyperemia and decreased lacrimation were consistent findings in man (Hepler, Frank and Ungerleider, 1972; Green, 1979). In dogs, several synthetic cannabinoids, administered orally, additionally produced conjunctivitis, keratitis, corneal opacities, and corneal ulceration (Thompson and Yang, 1976). These ophthalmic changes were postulated to be due to the reduced lacrimation and consequent dehydration of the corneal surface (Green, 1979).

The present investigation was undertaken to determine in the cat the ocular effects of Δ^9-THC as well as those of cannabichromene, a major cannabinoid occurring in some varieties of *Cannabis sativa*. Effects on IOP and ocular toxicity have been examined. In addition, the neurotoxicity of these two cannabinoids has been assessed in rats by evaluation of electrocorticographic activities after systemic administration.

2. Materials and Methods

A total of 50 adult cats of either sex weighing between 2·3 and 3·6 kg were utilized in these experiments. In order to facilitate drug administration, nictitating membranes were routinely removed surgically several weeks prior to the experiments. IOP was measured with a pneumatic tonometer (Alcon Laboratories, Fort Worth, TX) that had been calibrated against eyes of anesthetized cats by open stopcock manometry. The correlation coefficient for pressures up to 30 mmHg was 0·987. All animals were exposed to the experimental procedure for pressure measurement several times before initiation of the studies.

In experiments on acute cannabinoid effects on ocular tension, baseline pressure measurements were obtained both 1 hr and immediately prior to cannabinoid or vehicle administration. IOP was then measured once hourly for 6 hr. Δ^9-THC and cannabichromene, both dissolved in ethanol, were obtained courtesy of the National Institute on Drug Abuse. After evaporation of the ethanol under a jet of nitrogen, the cannabinoids were dissolved in light paraffin oil, saybolt viscosity 125/135 (Fisher Scientific Co., Pittsburgh, PA). Cannabinoids or vehicle were applied to the cornea in 25–50 μl volumes.

In experiments on chronic cannabinoid effects, cannabinoids or vehicle were delivered to the eyes of cats via osmotic minipumps and connecting extraocular cannulas. The cannulas were fabricated from polyethylene tubings of several diameters and silastic medical-grade tubing (Dow Corning) by fusion with a warm jet of air. Osmotic minipumps (Alza Corporation, Palo Alto, CA) devised to deliver solutions at a rate of 1 μl per hour over a period of nine days were utilized. Minipumps and cannulas were filled with either 2 % solutions of Δ^9-THC or cannabichromene or the vehicle, polyethylene glycol (PEG)-400; the latter vehicle has been demonstrated to function effectively in osmotic minipumps (Will, Cortright and Hopfer, 1980).

Cannula and minipump implantation were performed with the cats under anesthesia induced by sodium pentobarbital, 35–45 mg kg^{-1} intraperitoneally, supplemented with ketamine hydrochloride (Ketalar®, Parke–Davis, Detroit, MI), 10–20 mg kg^{-1} intramuscularly, as needed. The minipump with flow modulator was inserted subcutaneously in the neck region. The connecting cannula was then threaded subcutaneously from the back of the animal to above the eye with the use of metal guide tubing. The silicon tubing tip of the cannula was positioned on the cornea 5 mm central to the limbus.

IOP was measured at least once daily up to 11 days after minipump implantation. Animals were observed daily for any grossly visible signs of ocular irritation or corneal changes. Eyes were examined under a slit lamp and with an indirect ophthalmoscope at the end of the ninth day, at which time the minipump was removed.

A total of 18 adult male Sprague–Dawley rats weighing 200–300 g were utilized in the neurotoxicity studies. The technique employed previously in our laboratory for continuous monitoring of electrocorticographic and electromyographic activities was utilized (Colasanti and Khazan, 1975). Δ^9-THC (5, 10 and 20 mg kg^{-1}), cannabichromene (10, 30 and

100 mg kg^{-1}), or the PEG-vehicle (1 cc kg^{-1}) was administered intraperitoneally. Polygraphic recordings were monitored before and up to 24 hr after each injection.

Changes in gross ECoG activities were assessed, and evaluations of sleep and REM sleep parameters were undertaken, as described in previous studies (Colasanti and Khazan, 1971; Colasanti and Khazan, 1975). Statistical analysis of these data, as well as the remainder of the data in these studies, was undertaken with the utilization of Student's t test.

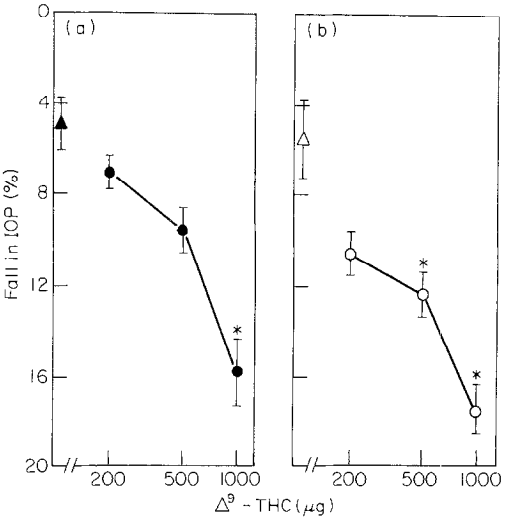

FIG. 1. Fall in IOP after unilateral topical application of Δ^9-THC to eyes of cats. (a) Treated eyes: ●, Δ^9-THC; ▲, mineral oil vehicle. (b) Untreated contralateral eyes: ○, Δ^9-THC; △, vehicle. Values are the mean ± S.E.M. for four to eight eyes. Absolute values of IOP prior to treatment ranged from 21·9 ± 1·04 to 24·2 ± 0·63 mmHg.

3. Results

Cannabinoid effects on IOP

After acute administration of Δ^9-THC to the eyes of cats, a dose-related fall in IOP occurred (Fig. 1). A trend toward pressure reduction was usually apparent 1 hr after treatment, and the duration of the effect ranged from 3 to 5 hr. The fall in IOP was quite small in magnitude and differed significantly from values for the mineral oil vehicle controls only at the highest dose (1000 μg). Untreated contralateral eyes showed falls in pressure comparable to those occurring in the THC-treated eyes (Fig. 1).

By contrast during the nine days of chronic administration of Δ^9-THC (20 μg hr^{-1}) via osmotic minipumps with connecting extraocular cannulas, IOP of the treated eyes remained 6–8 mmHg lower than that of the untreated contralateral eyes (Fig. 2). This pressure difference disappeared by the second day after minipump and cannula removal. Ocular tension of the PEG-vehicle-treated eyes (i.e. control group) generally ranged 1–2 mmHg lower in pressure than the untreated contralateral eyes during the same nine-day period (Fig. 2).

After acute administration of cannabichromene to the eyes of cats at doses ranging from 250 to 1000 μg, IOP was not significantly lowered (Table I). In fact, a trend toward pressure elevation was apparent after the two higher doses. After chronic administration of cannabichromene (20 μg hr^{-1}) for nine days, the pressure differences between treated and untreated eyes did not significantly differ from those observed after chronic administration of the PEG-vehicle (Fig. 3).

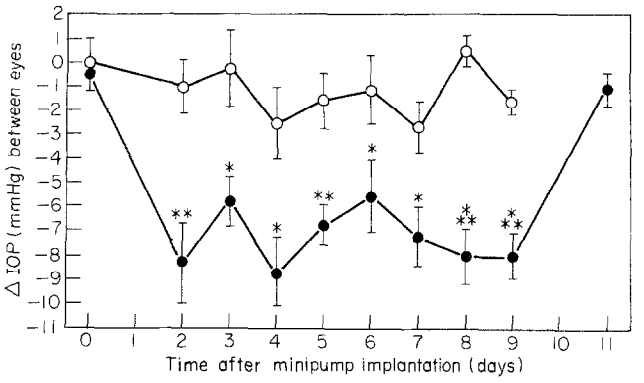

FIG. 2. Differences in IOP between treated and untreated contralateral eyes of cats after chronic administration of either Δ^9-THC (●) or the PEG-vehicle (control; ○), both of which were delivered via osmotic minipumps and connecting extraocular cannulas. The vehicle flow rate was 1 μl hr^{-1}, while the Δ^9-THC dose amounted to 20 μg hr^{-1}. Values are the mean ± S.E.M. for six to eight eyes. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, significantly different from corresponding value for vehicle controls.

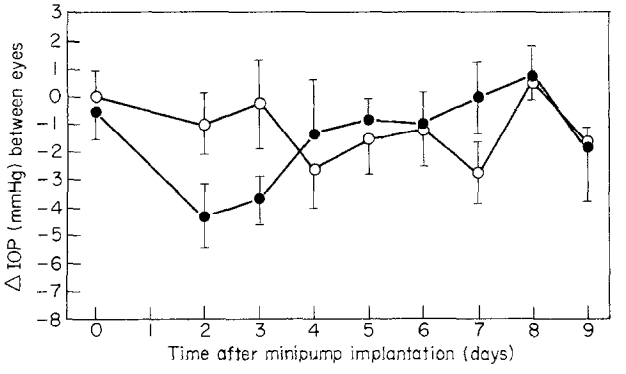

FIG. 3. Differences in IOP between treated and untreated contralateral eyes of cats after chronic administration of cannabichromene (●) or the PEG vehicle (control; ○), both of which were delivered via osmotic minipumps and connecting extraocular cannulas. The vehicle flow rate was 1 μl hr^{-1}; the cannabichromene dose was 20 μg hr^{-1}. Values are the mean ± S.E.M. for six to eight eyes.

TABLE I

Change in IOP after acute topical application of cannabichromene in the cat

	Change in IOP (%)			
	Cannabichromene (μg)			Vehicle (μl)
	250	500	1000	50
Treated eye	−5·6 ± 0·9	+2·5 ± 1·3	+7·3 ± 1·8	−5·4 ± 0·6
Contralateral	−3·8 ± 0·6	+5·0 ± 1·4	+4·2 ± 1·5	−2·8 ± 0·8

Ocular toxicity

Ocular examination of cats treated topically with either the PEG-vehicle or cannabichromene was unremarkable. Slit-lamp examination and indirect ophthalmoscopy revealed no abnormalities, and the ocular media appeared clear (Fig. 4). In

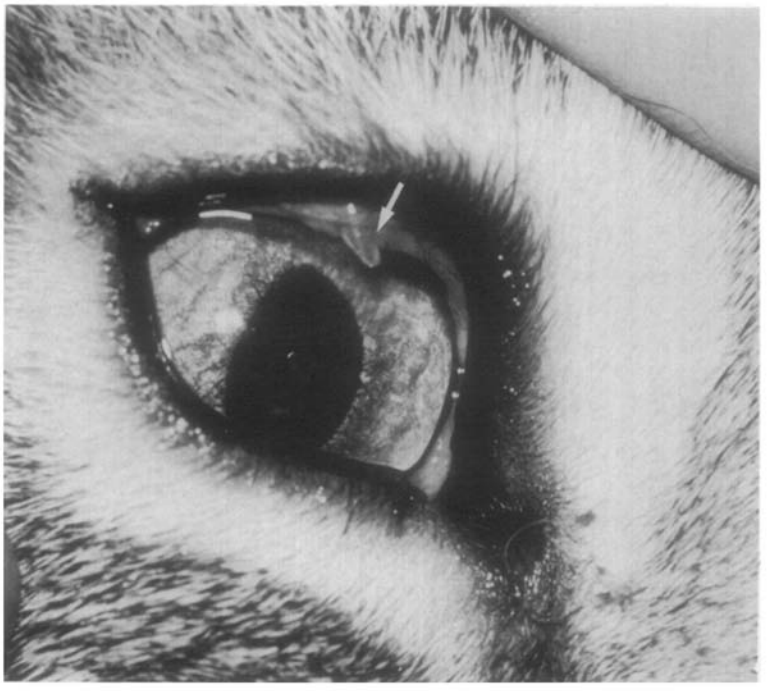

FIG. 4. Photograph representative of cat eyes treated topically with either the PEG-vehicle or cannabichromene. Arrow points to tip of the cannula connecting to the osmotic minipump.

contrast, eyes treated with Δ^9-THC developed marked chemosis, hyperemia, and erythema of the conjunctiva (Fig. 5). These changes first became apparent by the second day of THC administration. Grossly visible corneal opacities subsequently appeared between four to six days after initiation of the chronic treatment. These were preceded by irregularities of the corneal epithelial surface which were recognized as early as the third day. The opacities were reversible, with clearing occurring within a week after minipump and cannula removal.

Neurotoxicity

After intraperitoneal administration of Δ^9-THC to rats at doses of 5, 10 or 20 mg kg^{-1}, polyspike discharges 8–13 Hz in frequency appeared in the ECoG (Fig. 6) during wakefulness and behavioral depression of the animals. These polyspike bursts subsequently reappeared during the REM sleep episodes. The total duration of the appearance of the polyspikes within the awake state ECoG was dose-related (Fig. 7). The onset of sleep after the Δ^9-THC injections was significantly prolonged after the two higher doses in comparison with values for the PEG-vehicle-treated controls, and the onset of REM sleep was delayed after all three doses (Table II). REM sleep time for the 24-hr period after the injections was also significantly reduced after the highest dose.

After intraperitoneal administration of cannabichromene to rats at doses of 10, 30 or 100 mg kg^{-1}, ECoG and EMG activities did not differ in appearance from those preceding the injections or those for vehicle-treated controls. Sleep onset after the injections and REM sleep time within the first 24 hr were likewise not significantly different from values for the controls (Table II). The onset of REM sleep was significantly delayed only after the highest cannabichromene dose.

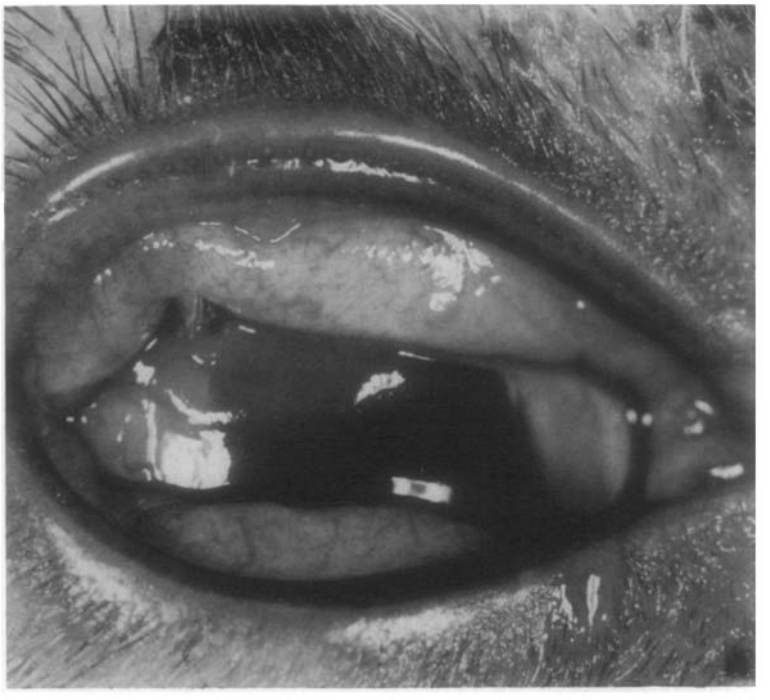

FIG. 5. Photograph representative of cat eyes treated topically with Δ^9-THC.

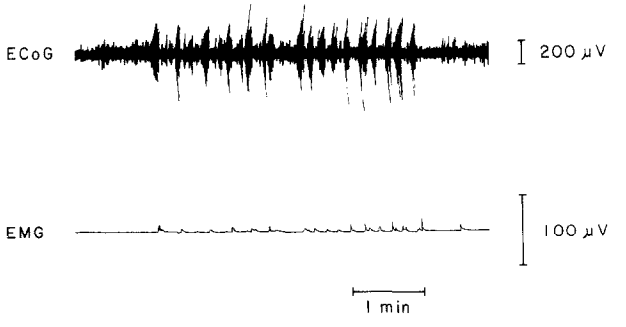

FIG. 6. ECoG and EMG tracings recorded 30 min after i.p. administration of $\triangle^9$-THC, 10 mg kg^{-1}, to a rat.

4. Discussion

The results of this study indicate that, as in the rabbit (Green and Pederson, 1973), dog (Merritt et al., 1981a,b), monkey (Green and Kim, 1977), and man (Purnell and Gregg, 1975), Δ^9-THC administered acutely lowers IOP in the cat. As in most other species studied, the magnitude of this effect was small, and a contralateral effect in the untreated eye was prominent.

In contrast with results after acute administration, the fall in IOP after chronic administration of Δ^9-THC to eyes of cats was quite substantial, amounting to 5–7 mmHg after deduction of the effects of the vehicle. The ocular cannula connected to the drug reservoir was quite effective in minimizing systemic absorption, as contralateral effects were minimal. Although the treated eyes were continuously

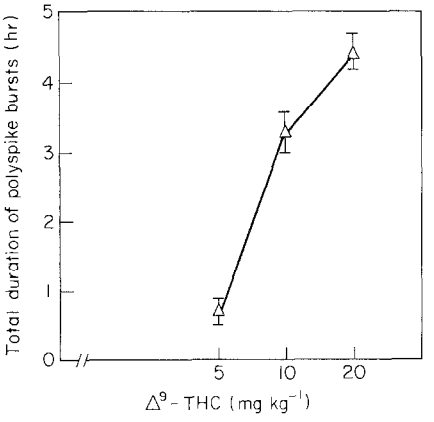

FIG. 7. Total duration of appearance of polyspike discharges in the ECoG during wakefulness after i.p. administration of Δ^9-THC to rats. Values represent the mean ± S.E.M. for four to six animals.

TABLE II

Effects of Δ^9-THC and cannabichromene on sleep and REM sleep parameters after i.p. administration to rats

	Δ^9-THC (mg kg^{-1})			Cannabichromene (mg kg^{-1})			Vehicle (mg kg^{-1})
	5	10	20	10	30	100	1
Sleep onset (hr)	1·0 ± 0·2	1·7 ± 0·4*	2·3 ± 0·2†	0·3 ± 0·1	0·5 ± 0·1	0·5 ± 0·1	0·4 ± 0·1
REM sleep onset (hr)	2·7 ± 0·5*	6·4 ± 1·4*	7·8 ± 1·1†	1·6 ± 0·5	1·7 ± 0·5	2·2 ± 0·2†	0·7 ± 0·1
REM time/24 hr (min)	79·6 ± 10·3	60·9 ± 20·6	45·6 ± 4·1*	98·6 ± 11·5	93·4 ± 6·3	78·3 ± 6·9	96·2 ± 12·2

* Significantly different from corresponding value for vehicle; $P < 0.05$.
† Significantly different from corresponding value for vehicle; $P < 0.01$.

exposed to Δ^9-THC around the clock, tolerance to the IOP-lowering effect did not develop by the ninth day of drug delivery. A lack of tolerance development to the pressure lowering effects of synthetic cannabinoids after twice daily application to rabbit eyes for one year has been previously reported (Green, Kim, Wynn and Shimp, 1977b).

The ocular toxicity observed after chronic treatment of cat eyes with Δ^9-THC was considerable. Conjunctival hyperemia was anticipated, as this phenomenon is a common occurrence in man in response to marihuana or Δ^9-THC (Hepler et al., 1972; Green, 1979). The appearance of corneal opacities, however, was not expected. Although this phenomenon was previously reported to occur in dogs after treatment with synthetic cannabinoids, it was considered to be species specific, as it did not occur in rats or rhesus monkeys (Thompson and Yang, 1976).

Δ^9-THC likewise produced considerable neurotoxicity after systemic administration to rats, with the production of rhythmic polyspike discharges initially during wakefulness and subsequently during REM sleep episodes. These polyspike bursts have previously been reported to occur after administration of marihuana extract, Δ^9-THC, or Δ^8-THC to rats (Lipparini, Scotti de Carolis and Longo, 1969; Masur and

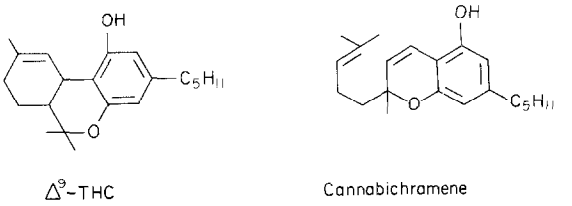

FIG. 8. Chemical structures of Δ^9-THC and cannabichromene.

Khazan, 1970; Colasanti and Khazan, 1971; Pirch, Cohn, Barnes and Barratt, 1972). They were not observed, however, after administration of cannabidiol (Colasanti and Khazan, unpubl.), a marihuana cannabinoid known to lack psychoactivity in man (Perez-Reyes et al., 1973; Hollister, 1974).

In contrast with Δ^9-THC, cannabichromene neither lowered IOP nor produced any overt ophthalmic changes after acute or chronic administration to cat eyes. This marihuana cannabinoid likewise did not produce any overt changes in the ECoG or behavior after systemic administration to rats, and REM sleep, a sleep stage readily disturbed by the administration of psychotropic drugs (Freeman, 1972), was not altered. As the major structural difference between Δ^9-THC and cannabichromene resides in the terpenoid ring (Fig. 8), these results suggest that it is this portion of the Δ^9-THC molecule that is involved in the mediation of the ocular and central effects of Δ^9-THC.

The results of this study have demonstrated that, in contrast with acute administration, continuous chronic administration of Δ^9-THC to cat eyes results in a substantial reduction of IOP. This pressure-lowering effect, however, is accompanied by considerable ocular toxicity and neurotoxicity. The terpenoid portion of the THC molecule appears to be important for the mediation of these effects.

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