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Intraocular Pressure, Ocular Toxicity and Neurotoxicity after Administration of Cannabinol or Cannabigerol

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Cannabinol or cannabigerol was administered to cats topically in doses of 250, 500 and 1000 μg as a single drop or chronically via osmotic minipumps (20 μg hr^{-1}) over a period of 9 days. While cannabinol had a modest effect on intraocular pressure after a single dose, it caused a more significant reduction in ocular tension during chronic administration. Cannabigerol had similar effects, but the magnitude of response to its chronic administration was greater. Cannabinol but not cannabigerol caused conjunctival erythema and hyperemia. After systemic administration of cannabinol (20, 40 or 80 mg kg^{-1}) to rats, 8–13 Hz polyspike discharges appeared in the electrocorticogram during wakefulness and during rapid eye movement sleep episodes. Cannabigerol (10, 30 and 100 mg kg^{-1}) lacked this effect. These results indicate that chronic administration of these cannabinoids lowers ocular tension considerably. Like marihuana and delta-9-tetrahydrocannabinol, cannabinol produced both ocular toxicity and neurotoxicity. As cannabigerol lacked these toxicities, it appears that the ocular hypotensive effect of this cannabinoid is somewhat dissociable from both the adverse central and ocular effects accompanying marihuana intake.

Key words: cannabinoids; cannabinol; cannabigerol; intraocular pressure; ocular toxicity; neurotoxicity; cats; rats.

1. Introduction

A lowering of intraocular pressure (IOP) in humans after smoking marihuana was initially observed by Hepler and Frank in 1971. Since then, studies in several species, including man, have demonstrated that the primary psychoactive marihuana constituent, delta-9-tetrahydrocannabinol (Δ^9-THC), has an ocular hypotensive effect (Green and Pederson, 1973; Purnell and Gregg, 1975; Green and Kim, 1977; Merritt, Peiffer, McKinnon, Stapleton, Goodwin and Risco, 1981). Two other psychoactive cannabinoids, delta-8-tetrahydrocannabinol and cannabinol, have also been reported to lower ocular tension modestly (Perez-Reyes, Wagner, Wall and Davis, 1976; Green, Wynn and Bowman, 1978). While cannabidiol, a major marihuana cannabinoid lacking psychoactivity (Perez-Reyes, Timmous, Davis and Wall, 1973), also modestly lowered IOP in rabbits (Green et al., 1978), this cannabinoid had minimal effect on ocular tension after intravenous administration to man (Perez-Reyes et al., 1976).

Ocular toxicity after administration of marihuana or Δ^9-THC has been reported in several studies. Both conjunctival hyperemia and decreased lacrimation were consistently observed in man (Hepler, Frank and Ungerleider, 1972; Green, 1979). Other toxic effects, including conjunctivitis, keratitis, corneal opacities, and corneal ulceration, were observed after oral administration of several synthetic cannabinoids to dogs (Thompson and Yang, 1976).

The present investigation of IOP and ocular toxicity was conducted to determine in cats the effects of cannabinol, a psychoactive marihuana cannabinoid (Perez-Reyes et al., 1973), and those of cannabigerol, a structurally-diverse naturally-occurring

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cannabinoid with unknown psychic effects. 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

IOP

The nictitating membranes of 55 adult cats of either sex (2·5–3·8 kg) were surgically removed to minimize interference with topical drug administration. Several weeks later, IOP was measured with a pneumatic tonometer (Alcon Laboratories, Fort Worth, TX) in preliminary sessions in order to accustom the animals to the procedure. The tonometer had been calibrated on anesthetized cannulated cat eyes by the open stopcock method (the coefficient of correlation between manometrically set and tonometrically measured pressure was 0·987 in the range 9–30 mmHg).

Acute experiments

IOP was measured at 1 hr and immediately before cannabinoid or vehicle administration and then at hourly intervals for 6 hr. Cannabinol and cannabigerol were obtained courtesy of the National Institute on Drug Abuse. Cannabinoids, dissolved in undiluted polyethylene glycol 400 (Sigma Chemical Co., St. Louis, MO) or vehicle, were applied bilaterally to the cornea in 25 or 50 μl volumes. Cannabinoid concentrations were 1, 2 or 4 %.

Chronic experiments

IOP was measured at least once daily for 12 days. Cannabinoids or vehicle were delivered to eyes via osmotic minipumps and connecting extraocular cannulas made by fusion of polyethylene tubings of several diameters and silastic medical-grade tubing (Dow Corning). The osmotic minipumps (Alza Corporation, Palo Alto, CA) delivered solutions at a rate of 1 μl hr^{-1} for 9 days. Minipumps and cannulas were filled with either 2 % solutions of each cannabinoid or the polyethylene glycol 400 vehicle; the latter vehicle was used because it is the only lipophilic solvent that has been shown to function effectively in osmotic minipumps (Will, Cortright, and Hopfer, 1980).

With the animals under anesthesia induced by sodium pentobarbital [35–45 mg kg^{-1} intraperitoneally (i.p.)] supplemented by ketamine hydrochloride [(Ketalar®, Parke-Davis, Detroit, MI) 10–20 mg kg^{-1} intramuscularly], minipumps were implanted as follows. One incision was made in the midline anterior to the scapulae and a second above the frontal bone. 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 metal guide tubing. The silicone tip was passed through the upper conjunctiva to lie over the eye through the lumen of a 16 gauge angiocatheter. The cannula was firmly anchored to underlying tissue. The silicone tubing portion of the cannula was then anchored firmly to the sclera, and the tubing was cut with a downward bevel so that the tip lay 5 mm central to the limbus. Finally, skin incisions were closed.

Ocular toxicity

Animals were observed daily for signs of corneal changes or ocular irritation. 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.

Neurotoxicity

Eighteen adult male Sprague-Dawley rats (200–300 g) were screened with a technique employed previously in our laboratory for continuous monitoring of the electrocorticogram (ECoG) and electromyogram (EMG; Colasanti and Khazan, 1975). Cannabinol (20, 40 and 80 mg kg^{-1}), cannabigerol (10, 30 and 100 mg kg^{-1}), or the polyethylene glycol vehicle

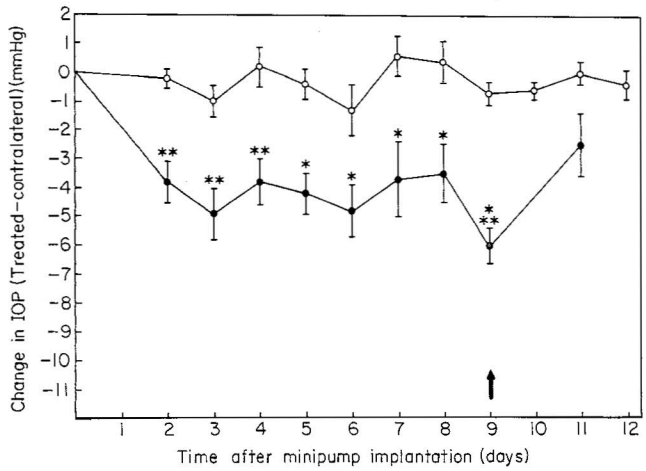

FIG. 1. Differences in IOP between treated and untreated contralateral eyes of cats after chronic administration of either cannabinol (●) or the polyethylene glycol vehicle (○), both of which were delivered via osmotic minipumps and connecting extraocular cannulas. The vehicle flow rate was 1 μl hr^{-1}, while the dose of cannabinol was 20 μg hr^{-1}. Baseline levels prior to minipump implantation were 20·0 ± 1·2 (vehicle treated) and 22·6 ± 1·6 (cannabinol-treated) mmHg. Depicted values are the mean ± S.E.M. for six to nine pairs of eyes. Arrow at day 9 indicates when the minipumps were removed. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, significantly different from corresponding value for vehicle controls.

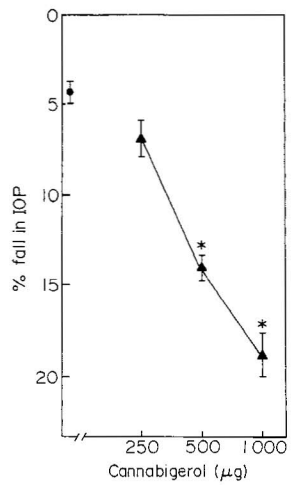

FIG. 2. Fall in IOP 3 hr after bilateral topical application of cannabigerol (▲) or the polyethylene glycol vehicle (●) to the eyes of cats. Values are the means ± S.E.M. for eight eyes. Absolute levels of IOP before cannabinoid or vehicle administration ranged from 17·0 ± 0·6 to 19·1 ± 1·0 mmHg. * $p < 0.05$, significantly different from vehicle control value.

(1 ml kg^{-1}) was administered i.p. ECoG and EMG activities were recorded before and up to 24 hr after each injection. Changes in gross ECoG activities were assessed, and evaluations of sleep and rapid eye movement (REM) sleep parameters were undertaken, as described in previous studies (Colasanti and Khazan, 1971; Colasanti and Khazan, 1975).

All data were statistically analyzed by Student's t test. A $p < 0.05$ was taken as the lower level of significance.

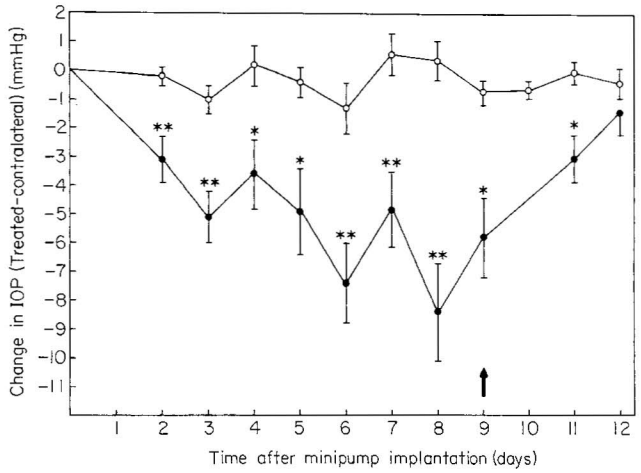

FIG. 3. Differences in IOP between treated and untreated contralateral eyes of cats after chronic administration of cannabigerol (●) or the polyethylene glycol vehicle (○), both of which were delivered via osmotic minipumps and connecting extraocular cannulas. The vehicle flow rate was 1 μl hr^{-1}; the cannabigerol dose was 20 μg hr^{-1}. Baseline IOP levels prior to minipump implantation were 20·0 ± 1·2 (vehicle-treated) and 19·5 ± 0·9 (cannabigerol-treated) mmHg. Depicted values are the mean ± S.E.M. for six to nine pairs of eyes. Arrow at 9 indicates when the minipumps were removed. *$p < 0.05$; **$p < 0.01$, significantly different from corresponding value for vhicle controls.

3. Results

Cannabinoid effects on IOP

Three hours after bilateral topical application of a single 1000 μg dose of cannabinol, there was a significant (21·4 ± 2·3 %; $n = 8$; $p < 0.05$) reduction in IOP from the baseline value of 23·5 ± 2·5 mmHg in comparison with values for vehicle controls (4·1 ± 0·4 % fall, $n = 8$, from the baseline value of 18·7 ± 0·3 mmHg). The lower dose of 500 μg did not significantly change IOP.

Throughout the period of chronic administration, IOP of cannabinol-treated (20 μg hr^{-1}) eyes remained 4–6 mmHg lower than that of the untreated contralateral eyes, while IOP of the polyethylene glycol vehicle-treated (1 μl hr^{-1}) eyes (control group) ranged 1 mmHg lower or higher in pressure than the untreated contralateral eyes; these differences between experimental and control groups were statistically significant (Fig. 1). They disappeared by the second day after minipump removal.

After bilateral application of single doses of cannabigerol (250, 500 or 1000 μg), a dose-related fall in IOP occurred (Fig. 2). Throughout chronic administration, IOP of cannabigerol-treated (20 μg hr^{-1}) eyes remained 4–8 mmHg lower than that of the untreated contralateral eyes, and this difference was statistically significant (Fig. 3). Pressure differences between these pairs of eyes returned to vehicle control values by the third day after minipump removal.

Ocular toxicity

Ocular examination of cats treated topically with either the polyethylene glycol vehicle or cannabigerol was unremarkable. Indirect ophthalmoscopy and slit lamp examination, undertaken before minipump removal on day 9, revealed no abnor-

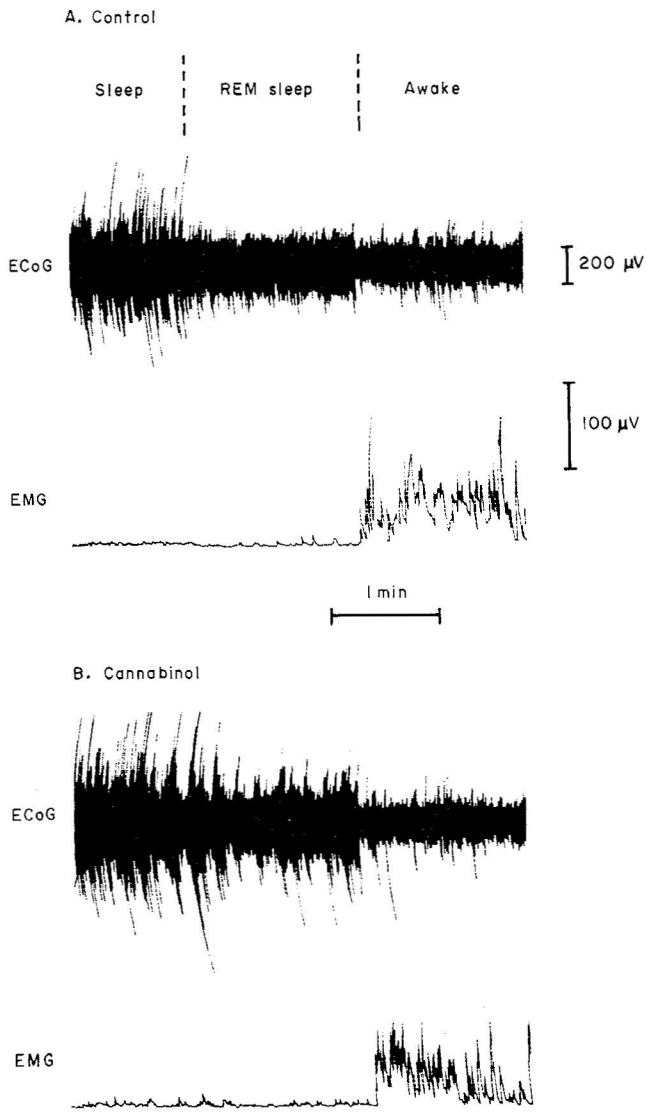

FIG. 4. ECoG and EMG tracings of the sleep-wakefulness cycle collected both 1 hr before (A) and 3 hr after (B) the administration of cannabinol (20 mg kg^{-1} i.p.) to a rat.

malities, and the ocular media appeared clear. On the other hand, eyes treated with cannabinol developed moderate chemosis, hyperemia, and erythema of the conjunctiva within 1–2 days. These changes remained apparent until after minipump removal.

Neurotoxicity

Within 10–15 min after i.p. administration of cannabinol to rats (20, 40 or 80 mg kg^{-1}), polyspike discharges 8–13 Hz in frequency appeared in the ECoG during wakefulness and behavioral depression. These polyspike bursts subsequently re-

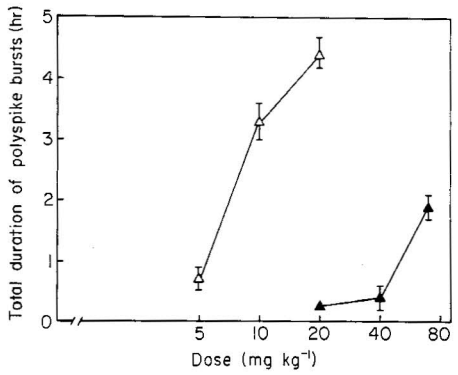

FIG. 5. Total duration of appearance of polyspike discharges in the ECoG during wakefulness after i.p. administration of either Δ^9-THC (△) or cannabinol (▲) to rats. Values represent the mean ± S.E.M. for four to six animals.

TABLE I

Effects of cannabinol on sleep and REM sleep parameters after i.p. administration to rats

	Vehicle 1 ml kg^{-1}	Cannabinol		
		20 mg kg^{-1}	40 mg kg^{-1}	80 mg kg^{-1}
Sleep onset*	0·4 ± 0·1	0·4 ± 0·2	0·5 ± 0·2	2·0 ± 0·9‡
REM sleep onset*	0·7 ± 0·1	2·5 ± 0·5‡	3·3 ± 0·8‡	10·4 ± 4·6‡
REM time (24 hr)$^{-1}$†	86·2 ± 9·3	88·2 ± 10·8	70·4 ± 11·4	6·9 ± 2·5‡

* Hours.
† Minutes.
‡ Significantly different from corresponding value for vehicle; $p < 0.05$.

appeared during the REM sleep episodes (Fig. 4). The total duration of their appearance within the awake state ECoG was dose-related but was shorter than that after Δ^9-THC (Fig. 5). The onset of sleep was significantly prolonged after the 80 mg kg^{-1} cannabinol dose in comparison with vehicle control values, and the onset of REM sleep was delayed after all three doses (Table I). REM sleep time was markedly reduced by only the 80 mg kg^{-1} dose.

For up to 24 hr after i.p. administration of cannabigerol to rats (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 controls. Sleep onset after the two higher doses, however, was significantly prolonged, and REM sleep onset was significantly delayed after all three doses (Table II). On the other hand, REM sleep time was not significantly changed.

4. Discussion

The results of this study indicate that, as in the rabbit (Green et al., 1978), cannabinol administered as a single dose causes a modest reduction of IOP in the cat. Cannabinol lowered ocular tension after chronic administration as well, and to a much

TABLE II

Effects of cannabigerol on sleep and REM sleep parameters after i.p. administration to rats

	Vehicle 1 ml kg^{-1}	Cannabigerol		
		10 mg kg^{-1}	30 mg kg^{-1}	100 mg kg^{-1}
Sleep onset*	0·4 ± 0·1	0·6 ± 0·04	0·9 ± 0·1‡	1·5 ± 0·3‡
REM sleep onset*	0·7 ± 0·1	1·7 ± 0·2‡	1·5 ± 0·1‡	2·2 ± 0·1‡
REM time (24 hr)$^{-1}$†	86·2 ± 9·3	85·6 ± 7·9	96·9 ± 16·2	85·6 ± 15·5

* Hours.
† Minutes.
‡ Significantly different from corresponding value for vehicle; $p < 0·05$.

greater degree than after single doses. Tolerance to this effect did not develop by the ninth (final) day of drug treatment.

As has been reported for Δ^9-THC (Hepler et al., 1972; Green, 1979), cannabinol caused moderate conjunctival erythema and hyperemia. On the other hand, although Δ^9-THC caused corneal opacities at the site of chronic drug delivery (Colasanti, Powell and Craig, 1984), cannabinol did not.

For simplicity in drug screening, neurotoxicity is usually measured as an impairment of observed spontaneous animal behavior, e.g. depression of locomotor activity or inability to remain upright on a rotorod. Electroencephalography provides a more sensitive analysis of neurotoxicity. It provides information on changes in spontaneous behavior during both wakefulness and sleep, and it also reveals any specific drug-related changes in ongoing electrical activity of the brain.

Polyspike discharges like those produced in the ECoG by cannabinol have previously been observed after administation of marihuana extract, Δ^9-THC, or Δ^8-THC to rats (Lipparini, Scotti de Carolis and Longo, 1969; Masur and Khazan, 1970; Colasanti and Khazan, 1971; Pirch, Cohn, Barnes and Barratt, 1972). In the present study, cannabinol was much less potent than Δ^9-THC in causing polyspikes, and the maximal effect of cannabinol was significantly lower (Fig. 5). These results in the rat correlate well with those obtained in man with regard to psychoactivity; the subjective effects of high intravenous cannabinol doses are reportedly much less intense than those of Δ^9-THC (Perez-Reyes et al., 1973).

Like cannabinol, cannabigerol modestly lowered IOP after topical application of single doses. IOP reduction was also greater after chronic administration. Tolerance to the ocular hypotensive effect did not develop by the final day of drug treatment.

Unlike cannabinol, cannabigerol did not cause ocular toxicity after chronic topical administration, nor did it overtly change ECoG or behavior after systemic administration to rats. In earlier studies in dogs or monkeys, two species showing characteristic behavioral responses to both marihuana extract and THC (Grunfeld and Edery, 1969; Mechoulam, Shani, Edery, and Grunfeld, 1970), overt behavior was also not altered by cannabigerol.

In the present study, cannabigerol did prolong the onset of both sleep and REM sleep. This finding suggests a slight stimulatory effect, although behaviorally the rats did not appear stimulated; instead, they showed intermittent writhing behavior for

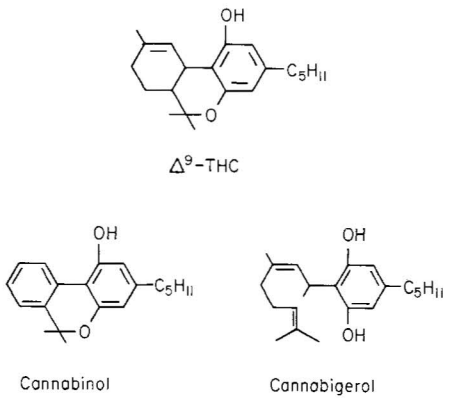

FIG. 6. Chemical structures of cannabinol and cannabigerol in comparison with that of Δ^9-THC.

more than an hour after injection. The delay in the appearance of sleep thus appears to be caused by peritoneal irritation rather than CNS stimulation. This possibility is supported by the finding of normal REM sleep time during the first 24 hr after injection, as REM sleep is a behavioral state easily disrupted by the administration of psychotropic drugs (Freemon, 1972).

Structurally, cannabigerol differs substantially from both cannabinol and Δ^9-THC in that two of the three rings present in Δ^9-THC are opened (Fig. 6). The ocular hypotensive effect of both cannabinol and Δ^9-THC, as well as that of marihuana, has unfortunately been accompanied by both ocular adverse effects and psychoactivity. Results obtained in the present study after administration of cannabigerol to cats indicate that the ocular hypotensive effect produced by this marihuana cannabinoid is dissociated from both the ocular toxicity and neurotoxicity accompanying marihuana intake over a relatively wide dose range.

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